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# PROTOPLASMA-MONOGRAPHIEN

VOLUME I

HEILBRUNN, THE COLLOID CHEMISTRY  
OF PROTOPLASM

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# Protoplasma-Monographien

Herausgegeben von R. Chambers (New York), E. Fauré-Fremiet (Paris), H. Freundlich (Berlin), F. E. Lloyd (Montreal), H. Schade (Kiel), W. Seifriz (Philadelphia), J. Spek (Heidelberg), W. Stiles (Reading). Redigiert von F. Weber (Graz) u. L. V. Heilbrunn (Woods Hole)

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VOLUME 1

## THE COLLOID CHEMISTRY OF PROTOPLASM

by

**L. V. HEILBRUNN**

Assistant Professor of Zoology  
University of Michigan

With 15 Illustrations

**Berlin**

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**1928**



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BY

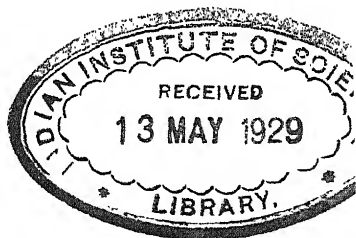
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TO  
MY FRIEND  
FRIEDL WEBER

1. The first part of the paper discusses the importance of the study of the history of the United States.

2. The second part of the paper discusses the importance of the study of the history of the United States.

3. The third part of the paper discusses the importance of the study of the history of the United States.

4. The fourth part of the paper discusses the importance of the study of the history of the United States.

5. The fifth part of the paper discusses the importance of the study of the history of the United States.

## PREFACE

This book was begun at the Kaiser Wilhelm Institut für physikalische Chemie in Berlin-Dahlem, it was continued at the Pflanzenphysiologisches Institut of the University of Graz, and it was completed at the Stazione Zoologica at Naples. Finally it was completely rewritten at Graz.

I have enjoyed the writing of it. Partly because of the kindly help of Prof. Weber and the pleasant discussions we have had together, partly because of the pleasure I have had in looking up old references to forgotten literature.

In present-day biology there is a tendency to avoid criticism. One does not like to hurt the feelings of one's fellow workers. And biologists seem particularly sensitive to criticism. When I began to write, I thought first that I would make no mention of work that I knew to be incorrect. It was Prof. Weber who urged me to discuss all papers good and bad. This in general I have done, although I have usually not attempted to consider the writings of those speculative authors whose work has been limited to an expression of opinion or the development of theory.

The modern biologist often reads only summaries or abstracts of papers. He scarcely has time to do more. As a result, he is apt to take an author's statements at their face value. Accurate trustworthy data receive no more attention than conclusions based on the flimsiest sort of evidence. Both are cited equally. The result is that our biological knowledge, and especially our knowledge of the physiology of the cell, is an unfortunate mixture of the true and the false. Perhaps, as some optimistic scientists insist, the true will eventually be sifted from the false. But why not hasten the sifting process? An incorrect observation or a wrong conclusion

may start many workers on false trails and may involve a huge waste of scientific time.

As one reads the literature of cellular physiology, as one runs across some incorrect statement cited over and over again, one often wonders if there might not be some means of preventing obviously false statements from finding their way into the literature. Perhaps, now and then, it might be possible for isolated workers to send their manuscripts away for criticism. I, for one, have frequently benefited by the critical help of others, and I should be only too glad to extend any critical help in my power to authors who might wish to send their manuscripts to me.

The science of the colloid chemistry of protoplasm is barely beginning. One could scarcely hope to develop the subject on a thoroughly logical basis. For this reason I have for the most part considered the experimental data piece by piece. Thus the book contains chapters on the effect of temperature, on the effect of acids, etc. Later, when the colloid chemistry of protoplasm is better understood, one may perhaps be able to write a book in which the subject is divided on the basis of the fundamental properties of the protoplasmic colloid, rather than on the basis of the type of treatment to which it is subjected.

Wherever possible, I have tried to indicate lines of research that might prove profitable. It is my hope that these hints may prove useful. Even in those cases in which I state that experiments of my own have been started in one direction or another, I do not feel that this should stand in the way of other workers who might wish to begin investigation in the same field.

The book was written during the tenure of a Guggenheim Fellowship. The author wishes to thank the donors of the fellowship for the opportunity of spending a year away from the demands of teaching, away too from the disturbances and distractions of American university life. Thanks are also due to Prof. Weber for the use of his splendid collection of reprints on the physical chemistry of protoplasm, to Prof. Linsbauer of Graz for the use of the library of the Pflanzenphysiologisches Institut, to Prof. Böhmig

of Graz for the privilege of using the library of the Zoologisches Institut, a library which is remarkably rich in the older literature, and to Prof. Freundlich and Dr. Ettisch of the Kaiser Wilhelm-Institut of Berlin for answering questions of a physico-chemical nature. Finally thanks are due to Frau Prof. Weber for the conscientious care with which she redrew those figures which were copied from other authors.

Graz, May 1928

**L. V. Heilbrunn**

For aid in correcting proof, I am especially indebted to Prof. J. W. Wilson of Brown University, and to Mr. L. G. Barth.

Woods Hole, September 1928

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## CHAPTER I

# INTRODUCTION

During the nineteenth century it became customary to think of living things as being made up of a special sort of material, the protoplasm. This might differ in various organisms, or in various parts of the same organism, but it was thought that all living substance, no matter where it was found, had certain characteristic properties not shared by the non-living. These characteristics were not usually stated in terms of the protoplasm itself, but rather in terms of vital function. Thus protoplasm could reproduce, it could grow, it breathed, that is to say it gave off carbon dioxide. In this way, the living properties of the organism as a whole were transferred to the stuff of which the organism was made. Although in some respects this is a useful point of view, it is far more important to be able to describe protoplasm directly in terms of what it is, rather than in terms of what it can do.

In books on biology, protoplasm is usually described as a viscous hyaline liquid. Probably it is a liquid, at least typically, although many recent authors have contended otherwise. To say protoplasm is viscous means little or nothing. Water is viscous in comparison with many other fluids, but in comparison with glycerine it is highly fluid. Speaking broadly, most protoplasm is quite hyaline, although the work of KEILIN '25 indicates a wide distribution of pigments in living cells.

The microscopic appearance of protoplasm has been the subject of countless studies, and the knowledge that has been built up has been gathered together in various books on cytology. Protoplasm is typically in the form of cellular units, each with a nucleus. The structures found within these cells are of different sizes and shapes, but on the whole there is a thorough-going

similarity in the cells of very different types of living material. In the next chapter some of the more essential facts regarding cellular morphology will be briefly enumerated. For the present let it suffice to say that morphological study has clearly demonstrated that living material, although never quite the same in different animals and plants, nevertheless presents a remarkable constancy of appearance when viewed under the microscope. Thus two animals of extremely different form and size are frequently found to have cells which are surprisingly similar.

Chemical study has shown this similarity to be more than a matter of form. Analyses of different animals and plants have yielded the same elements and the same types of compounds. There are differences to be sure. In some cases specific types of chemical compounds are found only in some organisms and not in others. But all living things are alike in that they are a salt water solution of proteins with an admixture of fats or lipoids.

It was in the nineteenth century that the underlying unity of life was first stressed. The cell theory and the evolution theory are only two aspects of such a point of view. More than anything else, it was this emphasis on the fundamental similarity of living organisms that made the development of theoretical biology possible. If each type of organism and each type of cell were totally unique, there would be a million biologies instead of a single science of biology, and the human mind could hardly attempt to solve such a vast myriad of enigmas. The knowledge that living things are remarkably similar has given the biologist courage to proceed with his investigations. Fortunately problems that are completely insoluble for certain types of organisms are relatively simple for others. And the results that have been gained for one type of living thing are often generally applicable. The laws of inheritance which were established for the garden pea are laws which apply to the inheritance of human characteristics. Ether, which was found to be anesthetic for human cells, is an anesthetic for most of the simpler organisms as well.

In stressing the similarity of living mechanism, it must not be forgotten that there are innumerable differences in various types of living material. It is true that there is one science of biology, but it is a science with infinite variations. Certain biological truths are well-nigh universal, others are relatively

specific. It is never safe to assume that what is true for one sort of protoplasm is true for another. Only experiment can decide. Sometimes what we might be inclined to regard as a general truth is extremely limited in its application, whereas another fact apparently specific may be found to apply to many different types of living substance. It is a natural tendency for a student of one particular sort of protoplasm to imagine that his results are of importance for every sort of protoplasm. The history of biological thought has shown the necessity of continual guard against such a tendency. Often enough what is true for the cells of one animal is not true even for the corresponding cells of a closely related animal.

There are facts about protoplasm which hold good for practically every type of living substance. And these are the facts which are most worth knowing. If it is true that all living cells manufacture carbon dioxide, in making this statement it is possible in a single sentence to give information regarding millions of types of protoplasm. This is far more important than to know that some special sort of cell is able to produce a specific chemical compound. The human mind can not hope to be all-knowing. We must cling to the facts which are broadly and generally true, and leave the specific details to gods and encyclopedias. But it is not merely a question of memory. Those characteristics of protoplasm which are universal are almost certain to be intimately related to the machinery of the living process, whereas a characteristic peculiar to a few cells must be related rather to the life of these particular cells than to life in general. The biologist whose primary interest is to gain an insight into the mechanism of life can well afford to neglect specific properties of certain types of protoplasm in focusing his attention on those properties which are universal.

Certain types of protoplasm are far more favorable for study than are others. Animals high in the evolutionary scale are made up of vast numbers of cells for the most part closely bound together. To be seen under the microscope these cells must usually be isolated, or at any rate cut up into smaller masses. Under these conditions abnormality or death frequently results. True, the newer methods of tissue culture have done much to make possible the maintenance of life in cells cut out from large masses of tissue, and perhaps in the future, cells in tissue culture will

more frequently be investigated by the student of living protoplasm. There are problems in the physiology of the cell for which the tissue culture method seems particularly suitable. Some types of cells are isolated in nature. Protozoan cells, yeast cells, the eggs of marine invertebrates, all these are familiar examples. An isolated cell is relatively easy to study. It must not be too small. With our present methods of study, no one could hope to learn much about the inner machinery of bacteria cells. On the other hand, not all large cells are favorable material. A bird's egg is made up mostly of yolk and investing membranes, only a small fraction of it is protoplasm, and what protoplasm there is can only be examined microscopically under conditions which involve the death of the cell. Protozoa have often been used in the study of protoplasm. Easy to obtain, in all seasons and in all places, the cells are often of a very convenient size for microscopic examination. Unfortunately many protozoan cells are not as simple as was formerly thought. They may be provided with neuromuscular systems or with various other miniature organs which give the protoplasm a high degree of specialization. Except for its food vacuoles and its contractile vacuoles, such organs are lacking in ameba, and it is thus easy to understand why this organism has so frequently been chosen for study by the student of protoplasm.

The egg cells of marine invertebrates have many advantages. They are large enough for thorough-going microscopic investigation, and yet often not so large as to be opaque. Many of them are quite transparent. Most invertebrate eggs are spherical. It is thus easy to estimate their volume. This is an important matter, for in many experiments it is essential that the volume of the cell remain constant. In any given species, the egg cells are usually of very uniform size. This is an advantage such material has in comparison with protozoan material, in which there is usually great variation in the size of individual cells. It is of course true that marine eggs can only be studied at certain stations and often only at certain times of year, but when the material is available, it is generally present in great abundance. Obviously not all marine eggs are equally valuable for purposes of study. Some worm and mollusk eggs are shed when they are in the metaphase of the first maturation division. If such eggs are subjected to almost any chemical or physical treatment

they immediately react by throwing off a polar body. It is therefore difficult to study the effect of chemicals on these eggs, for the experiment is usually complicated by the onset of the maturation process. For many purposes the sea-urchin egg is ideal. When shed or cut out of the ripe ovary, it is fully mature. Sea-urchin eggs can usually be obtained in large quantities, and the breeding season is very long, often lasting for many months.

Because of their many advantages, sea-urchin eggs have been widely used in protoplasm studies. Although these cells are devoid of all specialized organs and organelles, nevertheless it must be remembered that an egg cell is only one type of living substance and that any results gained with this sort of protoplasm can only provisionally be applied to other sorts of protoplasm. It is unfortunate that so few types of cells lend themselves to experimental study. The student of protoplasm should be ever on the lookout for new types of material.

For some purposes, the protoplasm of slime molds is especially useful. It occurs in large aggregates, and for this reason it offers unique opportunities for experiments on protoplasm in mass. Some authors have found the cells of various algae convenient for study. Algae often have very large cells, but in these instances most of the content of the cell is usually vacuole. This introduces complications, as does also the presence of a chromatophore like that of *Spirogyra*. Nevertheless the *Spirogyra* cell has frequently been used to advantage in protoplasm studies.

The cells of higher plants are also useful for many purposes. On the average these cells are larger than the cells of animal tissues, and most of them are relatively undifferentiated. In many plant cells the protoplasm is normally in a state of motion and this has often proven very helpful to the students of plant protoplasm. The speed of movement of the protoplasm is readily measured and may frequently serve as an index of changes in the physical properties of the living substance. The many studies of protoplasmic streaming which have been made in the past will be even more useful when a satisfactory mechanical explanation of the phenomenon is arrived at.

The first cytologists studied and described the morphology of protoplasm. Later, with the development of the science of

biochemistry, conclusions could be drawn regarding the chemical composition of the living substance. Until recently the physical properties of protoplasm have been field for speculation rather than for exact study. This is in part due to the intrinsic difficulty of making a physical study of such tiny objects as living cells. Chemical study is in some respects easier. One can analyze large masses of cells and be certain that the chemical compounds found in the entire ensemble are also present in the individual units. But many physical properties are not additive. The surface tension of a mass of cells is not the sum of the surface tensions of the single cells. The electric potential difference across a tissue can certainly not be calculated from the electrical potential difference across an individual cell, or vice versa. Generally if one is to obtain information concerning the physical properties of protoplasm one must study the individual cellular units. This is a broad statement and is not to be taken too exactly. Nevertheless, in the main, it is true.

To the biophysicist, protoplasm is chiefly interesting from the standpoint of mechanics. True, living cells give off heat and electricity, and some or perhaps all give off light radiations. But this energy is more in the nature of a by-product. Fundamentally the living cell is a machine which functions mechanically. The movement of a cell is essentially a problem in mechanics. When a cell grows, the primary physical factors, as far as we know, are such processes as diffusion and osmosis. A cell divides in a complicated mechanical fashion. Thus, from the standpoint of physics, it is the mechanics of protoplasm that is of the first importance.

In the physical study of the living cell, there have been two chief lines of investigation. Following the lead of PFEFFER, many authors have studied the osmotic properties of protoplasm. Cells are osmometers or osmotic machines, and they behave as such. In any osmometer the nature of the osmotic membrane is of primary importance. Within relatively recent years cellular physiologists have given a great deal of attention to the study of the permeability of the osmotic membrane of the living cell. There can be no question but that this is a subject that deserves full and careful study. Unfortunately, as is now generally recognized, many of the results that have been published do not stand critical examination. At present it seems just as important

to attempt an evaluation of the older experiments as to make new ones. From the large mass of papers that have been published, certain facts stand out fairly clearly. The relative ease with which different types of chemical compounds pass through the osmotic membrane of the cell seems to be quite well understood. On the other hand there is still a great difference of opinion as to the relation of permeability of the osmotic membrane of the cell to the activity of the protoplasm.

Important as are the osmotic properties of the cell and the cell membrane, they represent but one aspect of the physical study of protoplasm. The living substance is not a homogeneous material; beyond any question it is a colloid. In the study of colloids the physicist and the physical chemist have gathered together a vast body of information which forms a definite branch of physics and is called colloid chemistry. It is possible to study the colloidal behavior of protoplasm as well as its osmotic behavior. We are thus led to a colloid chemistry of protoplasm.

What is colloid chemistry and what are colloids? The definitions are somewhat arbitrary. When one substance is mixed with another its molecules may be scattered in such a fashion that each molecule is distinct. Or the molecules may form larger aggregates. Generally speaking when one component or substance is scattered through another so that the individual particles are larger than  $1\text{ }\mu\mu$  ( $0.000001\text{ mm.}$ ) and smaller than  $200\text{ }\mu\mu$ , the particles are said to be colloidal particles, and the entire mixture is spoken of as a colloidal solution. Colloid chemistry is the science of colloidal solutions, solutions whose dispersed particles have dimensions between  $1\text{ }\mu\mu$  and  $200\text{ }\mu\mu$ . Some substances of high molecular weight, for example proteins, have molecules so large that the individual molecules may be regarded as colloidal particles. The upper limit of size of colloidal particles,  $200\text{ }\mu\mu$ , is a purely arbitrary one. Ordinarily particles smaller than  $200\text{ }\mu\mu$  in diameter can not be seen with the microscope, and it is for this reason that this particular dimension has been chosen. But colloid chemists do not take the definition of their science too seriously. In general, when a material is dispersed so that its particles are larger than molecules or smaller than  $200\text{ }\mu\mu$ , the resultant solution has certain typical properties which are not found in true solutions or in very gross suspensions.

But there is nothing magical about these size limits. A suspension with particles just barely visible under the microscope differs in no way from one with particles just too small to be visible.

Broadly speaking, a colloidal solution has dispersed particles large enough so that they show the properties of matter in mass rather than the properties of individual molecules. Frequently the colloidal particles of any one chemical substance tend to adsorb at their surfaces traces of other materials, and these added materials often have a decided effect on the physical behavior of the entire particle. The entire particle with its adsorbed substances is sometimes referred to as a micella.

From the time that colloid chemistry first appeared as a science, no one has doubted that protoplasm is a colloid. The very fact that it is largely composed of proteins indicates that it must have colloidal properties. Materials of biological origin have always occupied a prominent position in the science of colloid chemistry. Milk and blood are colloids, so too is a solution of egg albumin. Gelatin, so often studied by colloid chemists in recent years, is a derivative of living tissue.

Many biologists have stressed the importance of developing a science of the colloid chemistry of protoplasm. But progress has not been rapid. For a long time, ideas with regard to the colloid chemistry of protoplasm were of two sorts. In the first place, it has been common practice among physiologists to explain every biological process in every sort of tissue by some postulated colloidal change in the protoplasm of the tissue cells. Such philosophical speculation was indulged in by the best and most prominent physiologists of the latter half of the nineteenth century; and as a matter of fact, this type of explanation still remains very popular in certain branches of physiology.

A more scientific, though hardly a more successful method of attack, was the method of analogy. Various inanimate colloids were, for one reason or another, chosen as being similar to protoplasm. The colloidal behavior of these relatively simple colloids was then studied as though it were the behavior of protoplasm itself. In the absence of other methods of study there is some justification for this type of work. Unfortunately the colloids chosen as models of protoplasm were extremely unlike the living substance. Consider gelatin for example. More conclusions have



been drawn about the colloidal properties of protoplasm from a study of gelatin than from a study of any other colloid. And yet in its fundamental behavior gelatin is entirely different from animate material. From the first it has seemed certain that protoplasm is coagulated by heat, and recent direct evidence supports this general notion. On the other hand, gelatin is liquefied by heat. Moreover gelatin solutions are gels at room temperature, whereas the types of protoplasm about which we have most certain knowledge are not gels.

If there is to be a colloid chemistry of protoplasm, it must be based on a direct study of the living substance itself. Life is undoubtedly dependent on the colloidal properties of the materials of which living things are composed. No amount of study of dead and inanimate substances will ever reveal these properties. Such study may eventually prove helpful. In the future it doubtless will. But as the study of the colloid chemistry of protoplasm makes its first crude beginnings, it is hardly possible to decide just which inanimate colloids are similar to the colloids of living cells. That is to say, similar in their physical properties. And all we can hope for at best is a remote resemblance. The simplest protoplasm is vastly more complex than any known inanimate colloid or mixture of colloids.

Only in the past ten or twelve years has there been any systematic attempt to study the colloid chemistry of protoplasm. Before this time there were a few sporadic observations, by-products of studies in other directions. And of course there were plenty of theories. But the investigation of the colloidal properties of protoplasm could not make progress until methods of work were established. The great difficulty in the way of studying the colloid chemistry of living cells lies in the fact that visual observation usually gives no certain information with regard to colloidal change. When gelatin in a test tube liquefies, this is immediately obvious even without measurement. The coagulation of egg albumin, or the flocculation of a colloidal solution of ferric hydroxide, can easily be followed with the naked eye. But when one looks at protoplasm, even with the highest powers of the microscope, it is usually impossible to detect even the most thorough-going change of state. True, authors in the past have described coagulations and liquefactions in living cells on the basis of simple microscopic observation. But the criteria

employed have often been purely arbitrary, and the results are frequently of little value.

That the physical state of the living colloid does undergo marked changes has been amply proven by recent studies. Moreover it seems certain that these changes in state are closely related to the fundamental machinery of the living process. From this standpoint alone, the colloidal study of protoplasm has made an auspicious beginning. But this is not all. Given the proper methods, the student of protoplasm can study it just as though it were an inanimate colloid, and in this way he can learn to know the physical properties of the most interesting of all fluids.

How can one study the colloidal properties of protoplasm? If microscopic observation does not show when the protoplasmic colloid changes from sol to gel, how is it possible to find out? In a test tube, when a colloid changes from sol to gel, it undergoes a great increase in viscosity and at the same time it becomes elastic. If then we were able to measure either the viscosity or the elasticity of protoplasm, we would have very definite information with regard to colloidal change. At the present time it is possible to make measurements of the viscosity of protoplasm, and these measurements can indeed be made with a fair degree of accuracy. Given such a possibility of measurement, it is no longer necessary to limit ourselves to speculation, or to confine our studies to inanimate colloids which are assumed to resemble protoplasm. We can actually begin a direct colloidal study of the protoplasm itself.

In a subsequent chapter the methods of viscosity measurement of protoplasm will be critically discussed. For the present let it suffice to state that such methods have been developed.

In studying the colloid chemistry of protoplasm it must not be forgotten that living cells are very small and that the conditions are thus different from those to which the colloid chemist is accustomed. When a small amount of electrolyte is added to a metallic sol, or when a protein is salted out, there is a precipitation of the colloid, and this is easily observable. But such a precipitation could hardly occur often in protoplasm, for a living cell may be much smaller than a single flake of a colloidal precipitate. The speed with which a precipitate settles depends on the size of the particles; for spherical particles it

increases as the square of the radius. Obviously in tiny cells there can be no rapid settling out of a precipitate, for the particles of a precipitate could never be large enough to settle rapidly. Thus in protoplasm one of the commonest types of colloidal change can not ordinarily be realized. It is not only in their small size that living cells differ from ordinary colloidal systems. Some of the peculiar characteristics of living colloids will be discussed in subsequent chapters.

Because of the fact that the conditions in living systems are so different from those of non-living systems, it is rather difficult to use the ordinary terminology of colloid chemistry. In general, it seems proper to refer to a very pronounced increase in viscosity, that is to say an increase of several hundred percent or more, as a gelation. In those cases in which the viscosity increase is certainly due to the appearance of a new phase, it seems logical to say that there is a coagulation. But it must be remembered that the suggested use of these terms does not exactly correspond to the manner in which they are used by the colloid chemist.

The colloidal study of protoplasm is not easy. Observations must be made on tiny droplets of a material incompletely defined from a chemical standpoint, and probably consisting of a mixture of highly complex compounds. The results that can be gained with such material must be regarded leniently. The biologist can not be expected to obtain data of the same degree of accuracy as those which the physical chemist can obtain with chemically pure compounds. But one must not be too lenient. If the colloid chemistry of protoplasm is to be a science, it must be based on firm facts and not on subjective impressions. In the chapters which follow, an attempt will be made to present the facts as they are now known. Few as these facts are, they show that a rational science of the colloid chemistry of protoplasm is possible and that it is now in the making.

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## CHAPTER II

# INTRODUCTION CONTINUED — THE MORPHOLOGY OF PROTOPLASM

Inanimate colloids may be studied in any container convenient for the experimenter. Protoplasm must be studied in cells. It will therefore be necessary to review briefly the elementary facts regarding the general form of cells, as well as the knowledge regarding the microscopical appearance of their contents. Colloid chemistry is directly concerned with the problem of form. In any study of a heterogeneous system, it is important to know the general arrangement of the various phases which compose it. In a colloidal solution, the size and shape of the dispersed particles are factors of importance. Inasmuch as colloidal particles are typically too small to be seen under the microscope, information concerning their size and shape can in most cases be obtained only by the use of some ultramicroscopic method. But, as was pointed out in the last chapter, systems whose dispersed phase is composed of particles barely visible with the microscope also show the properties of true colloidal solutions. Protoplasm contains many particles so small as to be barely visible. It is therefore important from the standpoint of colloid chemistry to determine exactly what can be seen when living cells are examined under the higher powers of the microscope.

The founders of the cell theory regarded the cell as primarily a receptacle with a wall. Later when it was found that some cells were apparently naked, the interior of the cell was thought to be more important, and a cell came to be defined as a mass of protoplasm rather than as a receptacle.

Every schoolboy now learns that a cell is a mass of protoplasm containing a nucleus. To be a discrete mass of protoplasm and

not an indefinable part of a whole, a cell which forms part of a tissue must have boundaries. In plant tissues, there is typically a fairly thick wall around each cell. When a piece of leaf or other plant tissue is placed in a concentrated solution of salt or sugar, the individual cells or protoplasts shrink away from their walls; they are then said to be plasmolyzed. In general, animal cells do not plasmolyze. When they are placed in concentrated solutions of salt or sugar, the entire cell shrinks and there is no wall outside of it. Apparently the animal cell is comparable to the plant cell without its wall.

The fact that living cells shrink in concentrated solutions and swell in very dilute solutions is regarded by most physiologists as evidence that the cell is surrounded by a semipermeable membrane. No other explanation seems to fit the facts, although a few unorthodox physiologists have contended that other explanations are possible. The semipermeable membrane which surrounds a living cell is called the plasma membrane. Physiologists often state that the plasma membrane is invisible, and this has become somewhat of a tradition. Actually there is no reason to suppose that the membranes which can be seen about animal cells are not plasma membranes.

The egg of the sea-urchin is surrounded by a hyaline membrane which appears to have very nearly the same refractive index as sea-water, and is thus hard to see. This membrane is about one micron thick. It is a semirigid structure, at any rate under certain conditions it will retain its form as the egg contents are squeezed out. When placed in a hypertonic solution, the egg decreases in diameter, and the membrane is pulled in with the shrinking protoplasm. Because of its rigidity, the membrane can be thought of as being under an elastic tension when it is pulled back in this fashion. There would then be a tendency for the membrane to spring away from the rest of the cell, and perhaps this occurs under certain conditions.

Many protozoan cells are surrounded by relatively thick membranes which are readily visible. In paramecium for example the outer membrane is a thick structure which bears cilia. That this rigid membrane is really the plasma membrane of the protozoan is indicated by the following argument. If we suppose a cell to be surrounded by a relatively rigid membrane, a membrane which could bend but which could not decrease its surface, shrink-

age of such a cell would not be possible unless the cell changed its shape in such a way as to have a smaller volume but yet the same extent of surface. This is exactly what happens when a paramecium is placed in hypertonic solutions of sodium chloride. Under these conditions the paramecium immediately flattens. The volume of the cell thus decreases, whereas the surface remains relatively constant, just as we have postulated for the case of a cell surrounded by a rigid membrane. There is thus reason to believe that the visible tough membrane of paramecium is its plasma membrane.

It may therefore be regarded as probable that the membranes which can be seen to surround some animal cells are the osmotic membranes of these cells. Perhaps in every case the visible membrane of the animal cell functions osmotically. In plant cells, on the other hand, it is generally impossible to distinguish a definite membrane inside the cell wall and separate from the main mass of the protoplasm. We conceive of a cell as a receptacle in which there is an outer membrane which functions osmotically. This membrane, the plasma membrane, may or may not be visible.

It should perhaps be stated that there are types of protoplasm which are really not in the form of cells. Some algae and many fungi consist of long strands of protoplasm with many nuclei. A muscle fiber also contains many nuclei. If we define a cell as a mass of protoplasm containing a nucleus, then a muscle fiber is either a group of cells without cell boundaries, or it is a single cell which differs from the usual type in that it possesses many nuclei. Cytologists usually refer to such a cell or group of cells as a syncytium. Obviously a syncytium is difficult to interpret in terms of the cell theory. Moreover it is only by a stretch of the imagination that tiny bacteria can be conceived of as cells. Books on cytology usually make no mention of bacteria.

Cells vary greatly in size and shape. Human red blood cells are discs the diameter of which is 7 microns. Sea-urchin eggs are spherical and in some species are about .75 microns in diameter. Cells which form part of a tissue mass may be prisms or polyhedrons. Animal cells are usually larger than red blood cells, and much smaller than sea-urchin egg cells. Of course there are exceptions. On the average, plant cells are larger than animal cells.

It has become part of the cytologists' credo to insist that every cell has a nucleus. In those cells which have no nucleus it is usually possible to distinguish granules which have the same affinity for dyes as do the granules of typical nuclei. Such granules are then referred to as a dispersed nucleus. Dispersed nuclei occur in a few protozoan cells, but are not ordinarily present in the cells of higher animals. Typically the nucleus appears as a spherical or ovoid body, and it is generally in the center of the cell. The volume of the nucleus may be as much as one-third of the volume of the cell, or it may be only one-hundredth of this volume or even less.

Concerning the form of the nucleus and the structures which can be observed in it both in the living and after treatment with various reagents, the cytologists have built up a large literature. When a cell divides, chromosomes are formed out of the nuclear material. For each species of animal and plant these have a typical number and often a particular shape or shapes. Volumes of description have been written about the chromosomes, and their study has proved one of the most interesting chapters of modern biology. Apparently the nucleus is the most important part of the protoplasm. It has, however, only seldom been studied from a physical standpoint. This is largely due to the fact that it is more difficult to obtain information concerning the physical properties of the nucleus than it is to obtain similar information concerning the cytoplasm.

With the first development of the microscope biologists began searching the living material for details of structure which might help them to understand its amazing properties. The microscopes of the seventeenth and eighteenth centuries were crude instruments, with lenses uncorrected for chromatic aberration. The early microscopist saw every object as a series of rainbows. More than anything else, it was the development of achromatic lenses in the early part of the nineteenth century that made possible the establishment of the cell doctrine and the growth of the modern science of cytology. But even before the appearance of the modern microscope, it was clearly recognized that the living substance contained granules. And just as every modern textbook of cytology or histology emphasizes the cell as the unit of the organism, so did the textbooks of the early nineteenth century emphasize the granule as the fundamental

unit of the living material (compare SCHLATER '99). This point of view has never quite faded out. There has been an unbroken line of investigators who have clung to the idea that the granules of protoplasm are the essential units. Many of these workers conceived of the granules as elementary organisms, in later years they have frequently been compared to bacteria. It has even been claimed from time to time that the granules are really capable of an independent existence outside of the cell.

Whereas to some authors the granules of the cell have seemed the only living part of it, others have held strongly to the view that the granules were of secondary importance, and that the life of the cell centered in the hyaline ground substance. In the lack of definite experimental evidence, neither view is justifiable. The centrifuge experiments of various embryologists interested primarily in the organization of the egg in relation to development\* indicate that various cells of developing invertebrate larvae can exist normally without the presence of one or another specific type of granule. In these experiments however, none of the cells is ever wholly granule free, partly because the granules are usually not completely moved by the centrifuge, but also because in most of these experiments the time between centrifugal treatment and cleavage is long enough to allow many of the smaller granules to return by Brownian movement to their original position in the egg. As a matter of fact, there seems every reason to believe that both granules and ground substance have a definite part to play in the life of the cell. Certainly as far as our present knowledge goes, we have no right to consider either the one or the other constituent as more truly living matter.

Interpretation aside, it is certain that the protoplasm of practically all cells contains vast numbers of tiny granules. These may be as much as three or four microns in diameter, usually they are smaller, and quite commonly they are almost at the limit of microscopic visibility. The granules are generally very numerous; there may be so many of them that the rest of the protoplasm is little more than a series of films surrounding the granules. The universal occurrence of granules in living cells

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\* For literature consult Morgan '27.



is certainly a fact which must be of great importance in any interpretation of the mechanics of protoplasm.

Some protoplasm contains fibrils or strands. In the eighteenth century the physiologist HALLER held the doctrine that all living things were made up of fibrils or "fibrae". This is a view that has reappeared from time to time in cytological literature. There can be no question but that muscles are largely made up of fibrils. The protoplasm of nerve fibers may also be fibrillar. Following the development of modern cytological technique, in which cells are treated with a variety of reagents before being sectioned, fibrils have been described in a great many types of cells. Whether these fibrils occur in the living cell is of course an open question.

From time to time in the history of cytology there have been arguments as to whether protoplasm was more truly granular or fibrillar, whether granules or fibrils were the ultimate units of the living material. It would scarcely be worth while to review these old arguments. As long as cytology remained a purely morphological study, it seemed of importance to discover some form element which could then be regarded as a unit, and in a certain sense as an explanation of protoplasm. There are types of living protoplasm which do contain fibrils. But ordinarily when living cells are examined under the microscope no strands or fibrils are visible. On the other hand, all or almost all living cells contain granules, and these are readily demonstrable without the use of any reagents or stains. Whereas fibrils are usually found only in highly differentiated types of living material, granules are of practically universal occurrence. In many cells the granules are extremely small, barely visible under the highest powers of the microscope. It is probable that in some protoplasm the granules may be still smaller and may thus not be detectable under the microscope. At least this much seems certain. Protoplasm is a colloid in which typically large numbers of the dispersed particles are of microscopic dimensions. In the language of the colloid chemist, it is a suspension.

In a given cell the visible granules are not always of the same type. Obviously in most instances it is not a simple matter to distinguish differences between granules so small as to be barely visible. Sometimes the granules are of two or more sizes. Thus in the egg of the sea-urchin *Arbacia*, in addition to granules

about a third of a micron in diameter, there are a micron in diameter. But the small granules of egg are also of two varieties. Most of them are heavier than the rest of the protoplasm, some are lighter. When centrifuged, the heavy granules go to one pole, the lighter to the opposite pole (see Fig. 1, p. 45). Many others have been shown to possess granules of fatty material which are lighter than the rest of the protoplasm. Perhaps in some cases these granules are really fluid and should be called droplets. If a particle is only a third of a micron in diameter, it is difficult to decide its physical state. The fact that granules of cells do not fuse when thrown to one side by centrifuge is evidence that they are solid, but it is not conclusive, for the failure of the granules to fuse might be due to the presence of a solid film. The small granules of the egg are certainly of two sorts, but there may really be many types of granules which with present methods we are unable to distinguish in the living cell.

Differences in size and specific gravity are the usual means of distinguishing granules in cells. Occasionally granules are obviously colored, but this seems relatively rare. For many years cytologists have been in the habit of identifying granules in cells by their staining reactions. In the classical cytology there was much discussion of the various types of granules which might be found in living cells. This is not so easily accessible, and it requires only brief mention at present. In general, staining reactions are apt to be deceptive, but in a few cases it is impossible to decide chemically by microscopic color tests. The same substance under varying conditions may or may not take a certain stain. The older cytological knowledge regarding the distribution of granules to be found in cells depends for the most part on observations made on dead, that is to say, fixed material. The use of metallic salts, alcohol, and various other reagents in order to make sections of cells possible. It is well known that such reagents are capable of producing granular precipitation in solutions of proteins. That they can also produce similar formation in living cells can be shown by a simple experiment. When a sea-urchin egg is centrifuged, some granules go to one pole, others to the opposite pole, and between the two

of granules is a hyaline region free from granules. On treating centrifuged egg cells with mercuric chloride, one of the common reagents used to fix cells, new granules appear in the hyaline protoplasm. It is therefore unsafe to draw conclusions about the granules of living protoplasm from the study of fixed protoplasm. This has been frequently recognized by cytologists and there has been an increasing tendency to compare fixed preparations with the living material wherever this is at all possible.

In 1897, the histologist BENDA coined the word mitochondria for certain types of granules which he found first in rat spermatoocytes and later in various other types of tissue. These granules often showed a tendency to form threads. BENDA developed a complicated fixing and staining technique which he believed to be specific for mitochondria. Later it was shown that the same granules could be demonstrated by much simpler methods, and that in many cases the mitochondria of BENDA were indistinguishable from the granules so often described by the older cytologists. At the present time there seems to be some slight uncertainty as to just what are to be termed mitochondria and in what respect they differ from the other granules of the cell. Some modern authors regard the dye Janus green as a specific test for mitochondria in living cells (see COWDRY '24). It is generally agreed that in most instances at least mitochondria in sectioned cells only appear when acetic acid and strong fat solvents have not been included in the fixing reagents with which the protoplasm was treated. RECAUD '08 and various other authors have expressed the view that the mitochondria contain a lipid constituent and that they are dissolved away by fat solvents. It might be possible to define mitochondria as granules composed in part of lipoids. This definition appears to have the advantage of precise statement. But it has not been universally accepted, at any rate lipid constitution is not regarded as an absolute criterion of mitochondria. Cytologists have identified certain types of granules as mitochondria mostly on the basis of their general behavior toward various fixing and staining reagents, but at best the mitochondria concept is not a very precise one.

In the *Arbacia* egg, the pigment granules or chromatophores stain selectively with Janus green (WILSON '26). They also disappear when the egg is treated with fat solvents. They thus

satisfy two criteria which in the past have been used to distinguish mitochondria. As a matter of fact it might not be inappropriate to regard these pigment granules as mitochondria, for in other eggs pigment granules have been regarded as mitochondria by mitochondria specialists (see DUESBERG '15, GATENBY '19). Curiously enough, although the pigment granules of the *Arbacia* egg disappear in the presence of fat solvent, this is not due to the fact that they are dissolved. In some recent studies, for the most part unpublished, the behavior of the pigment granules has been followed both inside and outside of the cells (see p. 229). Disappearance of the pigment granule in the presence of fat solvent is the result of a series of reactions, the first of which appears to involve the liberation of free calcium ion. If pigment granules in *Arbacia* are true mitochondria, it seems probable that the disappearance of mitochondria in fat solvents is not a simple solubility phenomenon.

In 1898, GOLGI described certain structures which appeared in nerve cells after these had been treated with a silver impregnation process. Following GOLGI, similar structures were found in various other types of cells, and many authors have described what is now generally known as a Golgi apparatus. This may take the form of a network or it may be granular. In every instance the Golgi apparatus is demonstrated either by silver impregnation or by an osmic acid treatment. Until recently the numerous descriptions of Golgi apparatus were all for fixed material, and there was no certain knowledge as to whether or not it might be present in living protoplasm. RAU, BRAMBELL, and GATENBY '25 and KARPOVA '25 describe Golgi apparatus in untreated cells.

The structures which can be seen only in fixed cells are relatively unimportant to the student of the mechanics of living protoplasm. When cells that are alive are observed under the microscope, they are seen to contain large numbers of granules. These may be of different sizes and of different chemical composition, but granules about half a micron in diameter are of very common occurrence. Such granules are found both in the cytoplasm and in the nucleus, although the nucleus contains relatively fewer granules, and may appear quite free from them. The fact that small granules are so constantly present in the living substance is an indication that such a fine suspension of material

represents a colloidal condition favorable for the life process. It seems certain that as the physiology of the cell becomes more clearly understood, there will be shown to be a definite dependence of many vital phenomena on the granular nature of protoplasm, on the properties which it possesses by virtue of the fact that it is a suspension.

It must not be forgotten, however, that there are outstanding differences between different types of living substance. When any particular type of cell is investigated from the standpoint of the colloid chemist, an effort should be made to determine what is visible in the cell under the microscope. Granules of one sort or another, fibrils if they are present, all would doubtless have an effect on the colloidal properties of the ensemble.

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### CHAPTER III

## INTRODUCTION CONCLUDED — THE CHEMISTRY OF PROTOPLASM

Colloid chemistry is a branch of physics. Through most of this book the discussion will center around the physical properties of the living substance. But before entering this field it may be well to review briefly the main facts about the chemistry of protoplasm. It will be necessary to consider not only the chemical constitution of the protoplasm, but also the reactions which occur in it. Naturally no exhaustive treatment is possible. Huge volumes have been written about certain aspects of the chemistry of protoplasm. In this chapter it will only be possible to outline a few of the essential facts, emphasizing here and there a point of especial interest to the student of protoplasmic mechanics.

Just as the colloidal properties of inanimate colloids are profoundly modified by traces of one substance or another, so beyond any question the colloidal properties of protoplasm are dependent in large measure on the ions and molecules which form a part of it. Moreover the intricate complex of chemical reactions constantly going on in the cell must both influence the colloidal condition of the protoplasm and be influenced by it. What these influences are we can for the present only vaguely hint at, but we can at least present the problems involved, as our modern knowledge shows them.

The student of the colloid chemistry of protoplasm is handicapped by the small dimensions of the cells which contain the material he is interested in studying. But this is not his only handicap. In ordinary colloid chemical investigation, the main mass of the material is a known substance or substances. True,

traces of unknown constituents may occasionally play a rôle in the colloidal behavior of an inanimate colloid, but in general the colloid chemist can be fairly certain of what his test tube contains. On the other hand the chemical make-up of protoplasm is extremely uncertain. We know a few broad facts and that is all. For the most part chemical analysis of the living substance is only possible under conditions in which the protoplasm is killed and doubtless changed during the course of the investigation. In general the ordinary analytical methods are too drastic. When such methods are used, the protoplasm dies, and as it dies a number of chemical reactions are initiated which may for all we know have a profound effect on the chemical compounds of which the cell is composed. To some extent these reactions of dying protoplasm can be retarded by cold, but they can not be completely stopped.

Some years ago it was not uncommon to regard protoplasm, or the essential living part of protoplasm, as a single chemical compound of extraordinary complexity. This view was thought to find support in the older experiments of HERMANN and PFLÜGER which seemed to indicate that the metabolism of the cell was bound up with very complicated systems, rather than with the chemical transformations of relatively simple compounds. The idea that a single chemical compound of extraordinary complexity was responsible for the life of the cell was elaborated by VERWORN '03 in his well known biogen hypothesis. VERWORN maintained that the biogen molecules were present in the ground substance of the protoplasm rather than in the formed elements such as granules, etc. It was not present in the nucleus, but the nucleus contained materials for the maintenance of biogen metabolism.

There is very little evidence in favor of the biogen hypothesis. In 1867, HERMANN concluded that muscular action depended on the breakdown and building-up of a complicated nitrogenous compound. This he thought of as a very labile substance, which only remains intact at 0° C, and which at room temperatures disintegrates into carbon dioxide, lactic acid, and myosin. HERMANN regarded this substance as of the same order of complexity as hemoglobin, that is to say he thought of it as a conjugated protein. But the recent remarkable advances in our knowledge of muscle metabolism have all tended to show that

relatively simple compounds may play a very important part in the chemical reactions which go on within the muscle. Thus carbon dioxide and lactic acid are formed from glycogen rather than from some complicated hypothetical substance.

Just as it is unwise to regard certain morphological elements of the protoplasm as more truly alive than others, so also it is unwise to maintain that certain of the chemical constituents of the protoplasm are more intimately concerned with the life process than others. Protoplasm contains many complex materials of high molecular weight, and there is always a tendency to ascribe the attributes of life to these less known and thus more mysterious compounds. But the simpler constituents are just as truly a part of the living system as are the complex. We can not conceive of a protoplasm without water and salts, and certainly these simple compounds form an indispensable part of the living material.

In order to have before us a picture of the chemical constitution of protoplasm, it will be necessary to review briefly the elementary facts concerning the chemical materials found in living cells. We will consider first the evidence obtained from the investigation of large masses of cells, and second the observations that have been made on individual cells in the hope of localizing definite compounds in various morphological elements of the protoplasm.

Water is the most abundant constituent of living material. In many types of living cells the water content is approximately 75 percent. Resting plant seeds have a much lower percentage of water, and certain animals, e. g. rotifers, can be dried until their water content is practically negligible. Such dried animals are scarcely alive, their protoplasm is in a quiescent state. As far as is known, active protoplasm is always rich in water.

Besides water, the only common inorganic constituents are the salts of the alkaline and alkaline earth metals. Below are given, first an analysis of the salts of maize pollen cells, and second a recently published analysis of the salts of the sea-urchin egg. These are merely two examples of many that might have been chosen. They represent analyses of cells rather than of tissues. This is an advantage in that tissues often contain lifeless connective tissue fibers, doubtless of very different chemical composition from the protoplasm itself.



## Composition of ash of maize pollen (ANDERSON and KULP '22)

Phosphorus . . . . .	18.92%	Magnesium . . . . .	4.60%
Sulphur . . . . .	0.69%	Potassium . . . . .	35.48%
Chlorine . . . . .	0.80%	Sodium . . . . .	0.69%
Silica ( $\text{SiO}_2$ ) . . . . .	3.76%	Iron . . . . .	0.25%
Calcium . . . . .	1.02%	Aluminum . . . . .	0.22%

Salt content of the *Arbacia* egg (Page '27)

Radical	Milligrams per million eggs	Millimols	Milli-equivalents
Calcium . . . . .	1.90	0.047	0.094
Magnesium . . . . .	4.48	0.182	0.364
Sodium . . . . .	1.301	0.056	0.056
Potassium . . . . .	2.445	0.063	0.063
Iron . . . . .	0.030	0.0005	0.0015
Total cation:		0.348	0.5785
Sulphate . . . . .	0.00046	0.00004	0.00008
Chloride . . . . .	0.1864	0.0053	0.0053
Total phosphate . . . . .	0.9064	0.0291	0.0873
Nitrate . . . . .	Trace	—	—
Total anion:		0.0344	0.0927

In the above analysis of the sea-urchin egg,  $\text{SiO}_2$  was found to be present, but was not determined quantitatively. No distinction was made between the phosphorus combined with lipid and the inorganic phosphorus, but PAGE indicates that about half of the phosphorus was inorganic. PAGE's table is especially interesting in that he has calculated the molar concentrations of the various ions. Apparently there is a much higher concentration of cation than anion within the cells. This also appears to be true for the pollen ash. If from PAGE's data one calculates the osmotic concentration of the salts within the sea-urchin egg, it is possible to show that this concentration is very much higher than the concentration of the salts in the surrounding sea-water.

The fact that some living cells and perhaps living substance generally contains more inorganic cation than anion is very important. In the sea-urchin egg PAGE found the molar concentration of cation to be ten times as great as that of anion. The

equivalent concentration was six times as great. Such an excess of cation may very well have a bearing on the colloidal properties of the protoplasm. The subject is in need of further elucidation. Doubtless some of the excess cation is combined with protein, some is probably combined with lipoid or fat. In the sea-urchin egg it is noteworthy also that there is a larger amount of bivalent cation (Ca and Mg) within the egg than monovalent cation (Na and K), and this in spite of the fact that the surrounding sea-water has only a relatively small amount of bivalent cation.

Analyses such as the above indicate the actual constitution of one type of cell or another. Obviously the inorganic salts which are found in a cell may vary somewhat as the environment of the cell or organism changes. By experimentally varying this environment it is possible to obtain data with regard to the salts which are indispensable for the protoplasm of a definite species of animal or plant. For the most part experiments of this sort have been made only with plants. Seeds can be grown in nutrient solutions which contain some elements and not others. In such nutrient solutions, if all the necessary elements are present, large normal-looking plants are obtained. On the other hand in the absence of some needed element, dwarfed pathological types appear. Details concerning these experiments can be found in most books on plant physiology. Flowering plants require the following ions, K, Mg, Ca, Fe,  $\text{NO}_3$ ,  $\text{SO}_4$ ,  $\text{PO}_4$ . Normal growth can be obtained without Na, Cl,  $\text{SiO}_2$ . In the studies that have been made with flowering plants some salts are of course contained in the seed, and this is a possible source of error. It is doubtful also whether the older experimenters were careful enough of the purity of the salts they used to make up their nutrient solutions. Often mere traces of a substance are important. Thus PETERS '21 found that the protozoan *Colpidium* was able to live in solutions which contained no potassium only if glass vessels were used. In quartz vessels the animals died. PETERS took this to mean that enough potassium dissolved out of the glass to keep the animals alive.

For bacteria, yeasts, and molds, it has been found that the only cations necessary are K, Mg and Fe, and there seems to be some question as to whether iron is essential or not. In experiments with these organisms, there is no source of error

from the presence of a large seed. However, there is always the danger of impurities.

The study of the elements which are required for animal life is often very difficult. The higher animals are easy to rear on a pure chemical diet, but they are large at birth, and it is quite impossible to decide whether or not an element is necessary unless large amounts of it are required. Sometimes this is the case, and many studies have been made on the need of such elements as calcium and iodine. In these instances, the calcium goes largely into the bones, and the iodine into the thyroid gland, so that such experiments are of little interest to the student of protoplasm, who is concerned chiefly with the inorganic needs which are common to cells in general. It seems not at all unlikely that experiments with lower animals might yield results of value. A few studies have been made with protozoa, and some work has also been done on *Drosophila*.

The organic materials found in animals and plants are of three main types; proteins, fats, and carbohydrates. To the fats should be added a heterogeneous group of fat-like substances, fat-like in view of the fact that they are generally insoluble in water but soluble in liquids of low surface tension such as ether and chloroform. These fat-like substances are called lipoids. Concerning the present-day knowledge regarding proteins, fats, lipoids, and carbohydrates, abundant information will be found in any one of numerous textbooks or handbooks of biochemistry. From a physico-chemical standpoint it seems especially noteworthy that all protoplasm should contain in addition to water-soluble substances others that are typically water-insoluble. We should expect that the fats and lipoids of living cells would often be found in the form of a suspension or emulsion. This apparently is true.

The following tables give analyses of plant and animal cells.

Composition of the egg of *Sabellaria alveolata* (FAURÉ-FREMIET '21)

Water . . . . .	70.00%
Proteins. . . . .	19.08%
Fats and lipoids . . . . .	6.80%
Glycogen . . . . .	1.27%
Ash . . . . .	1.53%
	<hr/> 98.68%

Composition in percent of dry weight of the pollen of maize  
(ANDERSON and KULP '22)

Nitrogen . . .	4.53%	Crude fiber . .	5.37%
Starch . . .	11.07%	Fatty material.	1.48%
Saccharose . .	9.09%	Ash . . . . .	3.46%
Pentosanes . .	9.60%		

In the pollen, the carbohydrates starch, saccharose, and the pentosanes represent about 30 % of the dry weight, whereas the amount of protein calculated from the nitrogen content is roughly 27 %. Thus in the plant cells there is more carbohydrate than protein, whereas in the animal cells there is almost fourteen times as much protein as carbohydrate.

In myxomycete protoplasm there is also a high percentage of carbohydrate. The following table, taken from a recent paper of KIESEL, shows almost as much carbohydrate as protein.

Chemical composition of *Reticularia lycoperdon* (KIESEL '25)

Oil . . . . .	17.85%
Lecithin. . . . .	4.67%
Cholesterin . . . . .	0.58%
Reducing carbohydrates . . . . .	2.74%
Not reducing, soluble carbohydrates (exclusive of glycogen) . . . . .	5.32%
Glycogen . . . . .	15.24%
Polysaccharide, hydrolyzable with difficulty . . .	1.78%
Extractives (containing nitrogen) . . . . .	12.00%
Proteins, including plastin . . . . .	29.07%
Nucleic acid . . . . .	3.68%
Oil of lecithoproteins (?) . . . . .	1.20%
Unknown substances . . . . .	5.87%
	<hr/> 100.00%

The chemical study of large masses of animate tissue or of large numbers of cells is relatively simple compared to the study of individual cells or the attempt to identify chemical compounds within the confines of a single cell. In general, insoluble constituents of protoplasm would tend to be more localized in their distribution. Obviously if a substance is highly soluble, we should expect to find it dispersed throughout the entire cell. But this need not necessarily follow. A soluble substance may be very

largely adsorbed at some of the protoplasmic surfaces. It has been pointed out that protoplasm is typically granular, and the surfaces of the granules no doubt adsorb many dissolved substances. Often a cell contains various types of granules, and it is quite possible that the adsorption at the surface of one kind of granule may be quite different from that at another.

In the attempt to identify chemical compounds within a living cell, one encounters a host of difficulties. The small size of the cell is not the only troublesome factor. Microchemical analysis has developed many methods for the identification of extremely small quantities of material. But microchemical analysis depends very largely in its identifications on the formation of characteristic crystals, which are then recognized under the microscope. Within a cell, or rather within the protoplasm, crystal formation is usually impossible. Generally speaking, normal crystals do not form in the presence of large quantities of colloidal material. And living cells contain high concentrations of various colloids. A few microchemical tests are color tests. Iron can always be detected by its color reactions. So too can starch and glycogen. These tests have been used on many living cells. Unfortunately the number of such tests is not very great, and not all of the tests that have been used are valid. Cytologists often assume without adequate proof that certain dyes are specific for definite chemical substances.

In addition to color reactions, the student of cell chemistry can also make use of solubility tests. Cells are treated with fat solvents, with salt solutions, or with various enzymes, and the disappearance of one structure or another is regarded as evidence of its solubility. Inferences are then drawn in regard to the chemical constitution of the structure which was found to disappear. Solubility tests on protoplasm are not as reliable as they might seem. In the last chapter an example was cited of certain granules which always disappeared in the presence of fat solvents, and which were apparently neither fats nor lipoids. The disappearance of these granules was the end result of a series of reactions initiated by the fat solvent. In a system as complicated as protoplasm the addition of a foreign chemical may produce results which are difficult to interpret. Doubtless, however, many of the results that have been obtained with solubility tests are valid. For further and more detailed information concerning



microchemical tests, see PARAT '27, compare also KLEIN '28, and PATZELT '28.

The microchemical study of the cell has among other things given information regarding the distribution of glycogen, of fats, and of nucleoproteins within the cell. It seems fairly certain that the nucleus consists largely of nucleoprotein. As a matter of fact nucleoproteins were first isolated from the heads of spermatozoa. Fats and lipoids are usually scattered through the protoplasm and are not limited to the surface layer of the cell nor even especially abundant there.

HEILBRUNN '26b has suggested that protoplasmic granules are typically surrounded by a lipoid or fatty film. The evidence for this view rests both on the colloidal behavior of the protoplasm and also on the fact that when sea-urchin eggs are centrifuged, the granules heavier than the rest of the cytoplasm give a reaction with osmic acid. This indicates the presence of fats or lipoids, with an admixture of unsaturated fatty acid, in the granular constituents of the protoplasm, and presumably at their surface.\* Further evidence that the granules of protoplasm are typically surrounded by a fatty or lipoid film is given by the fact that many types of protoplasm resist the action of pepsin and trypsin, but are soon digested if their fats are first extracted (for literature on this subject, see BIEDERMANN '24). According to BIEDERMANN, there are two possible explanations of this phenomenon. One might suppose that the lipoid was combined with protein in the form of a lipoprotein, or one might regard the lipoid as held by adsorption. BIEDERMANN states that the first possible explanation can not very well be the true one, because of the fact that lipoids can generally be completely removed from cells by lipoid solvents. There is therefore evidence from several directions in support of the view that protoplasmic granules are typically surrounded by a film of fat or lipoid. In subsequent

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\* It is possible that the protoplasm may contain lipoids with a relatively high content in phosphorus and a specific gravity greater than water. But it is doubtful if such lipoids could have a specific gravity greater than the specific gravity of the cytoplasm of the sea-urchin egg. The specific gravity of this cytoplasm must be over 1.03. Moreover the granules in question have a specific gravity of at least 1.10 (see Chap. 5), and it seems certain that no protoplasmic lipoids have a specific gravity as great as this.

chapters it will be shown that this conception of the protoplasmic granule fits in with certain of the known facts regarding the physical behavior of the living substance.

From the standpoint of cell chemistry, as well as from the standpoint of the colloid chemistry of protoplasm, one of the most important questions to be solved is the question as to the degree of acidity or alkalinity of the protoplasm. This problem is by no means a new one, but in recent years it has taken on a new significance.

Many authors have attempted to determine the hydrogen ion concentration of protoplasm. Their results have varied not only with the type of cell studied, but also with the method. For relatively simple systems under known conditions the most accurate method of measuring hydrogen ion concentration is the electrical method. The solution to be studied is connected with a hydrogen electrode on one side and some standard electrode such as a calomel electrode on the other. The E. M. F. of the cell is then measured, and from this E. M. F. the hydrogen ion concentration, or its negative logarithm, the pH, can readily be calculated. In determining pH electrically one must be very careful to exclude any factors which may have an influence on the E. M. F. For instance a hydrogen electrode may frequently serve as an oxygen electrode. Or various substances can "poison" the electrodes. The electrical measurement of the pH of protoplasm is complicated by the fact that it is very difficult to devise electrodes small enough to be inserted into a living cell. This technical difficulty has been overcome by several recent workers. Thus TAYLOR and WHITAKER '27 have succeeded in inserting electrodes into the protoplasm of *Nitella*. Their results however are very improbable and TAYLOR and WHITAKER do not regard them as a proper measure of the protoplasmic pH. BUYTENDIJK and WOERDEMAN '27 have devised an antimony electrode which they have introduced into frog eggs and similar objects. The results of BUYTENDIJK and WOERDEMAN are certainly more within the range of probability than those of TAYLOR and WHITAKER, but there is no means of knowing whether or not they are accurate. Many factors might influence the E. M. F. of a concentration cell, one end of which is immersed in protoplasm. When a cell is cut or torn, it frequently forms films or membranes at its free surface (see chap. 13). There is good reason to believe

that such a film or membrane might be formed about any object inserted into a cell, and the presence of a membrane might have a considerable influence on the E. M. F. of any current which had to pass through it. There are also certain difficulties connected with the use of the antimony electrode (see VLÈS and VELLINGER '27).

Some workers have ground up cells and have determined the pH of the resultant mass. In view of the many changes which accompany death, one would have to be rather optimistic to assume that the pH of living cells could be measured in this fashion.

Most of the measurements of protoplasmic pH have been made with indicators. If cells are placed in solutions of various indicators, the amount of dye which enters depends both on the permeability of the plasma membrane and on the readiness with which the protoplasm combines with the dye. An indicator may be either adsorbed or it may be bound chemically. Naturally the concentration of the dye within the cell will be very different from its concentration in the surrounding solution. The intensity of its color will therefore be quite different, and one is apt to make errors if one attempts to estimate pH from color intensity. Some authors have tried to control the amount of indicator in the cell by injecting indicators with micropipettes. Perhaps more reliable results can be obtained in this way, but it is always difficult to control the amount of dye injected, and there is always the chance of violent injury.

It seems obvious that the most reliable results can be obtained with indicators that undergo a complete change of color, rather than with those that merely change their shade. An indicator may be light red or dark red either because of a difference in pH or because of a difference in concentration of the indicator; and the concentration of the indicator is not known. But when an indicator changes from red to blue, the concentration does not so much matter. For this reason some investigators have emphasized the importance of using indicators which change color, and it is obviously an advantage to use as many different indicators as possible. This point of view has been most often stressed by SMALL and his collaborators (see SMALL '26, REA and SMALL '26 and various other papers in volumes I, II and III of *Protoplasma*). Unfortunately SMALL and his co-workers have used alcoholic



solutions of indicators, so that their results are valid for dead cells rather than for living cells. SMALL claims that his results can be duplicated with aqueous solutions of the indicators, but apparently many or most of the dyes he uses are strongly toxic, so that even in aqueous solution they kill the cells. Since the above was written, SMALL has recognized the error due to the presence of alcohol, and has attempted to devise a new and improved technique (see INGOLD and SMALL '28).

In view of the uncertainty with regard to the various methods which have been used for the determination of the pH of living cells, it is difficult to decide which values are the more likely to represent the nearest approach to the truth. In their work on the starfish egg, CHAMBERS and POLLACK '27 used a number of indicators and observed color changes rather than mere difference in intensity of shade. They found the pH to be 6.7. Indeed many workers have found the pH of living cells to lie somewhat on the acid side of the neutral point. However the results of various investigators are far from concordant, and we have as yet no means of knowing whether this discordance is due more to differences in the hydrogen ion concentration of different types of protoplasm, or to errors in method and observation. For references to literature on intracellular hydrogen ion concentration, see PFEIFFER '26, REISS '26.

An interesting recent paper is that of VLÈS and VELLINGER '28. They used the natural pigment of the *Arbacia* egg as an indicator and they were thus able to determine the pH without crushing the eggs and without injuring them by microdissection. Examined spectroscopically the pigment of the *Arbacia* egg shows different absorption bands at different hydrogen ion concentrations. From their studies VLÈS and VELLINGER concluded that the pH of the *Arbacia* egg cytoplasm in the neighborhood of the pigment granules was between 5.2 and 5.9.

In all types of protoplasm there are many and complicated chemical reactions. Of these reactions the ones most often studied are the oxidation-reduction reactions. From a physico-chemical standpoint, oxidation involves the loss of an electron or electrons from a molecule. By the study of electric oxidation-reduction cells, it is possible to express the tendency for a substance to become oxidized or reduced in terms of electrical units. The measure of this tendency is the oxidation potential. It is to be hoped that

in the future accurate determinations of the oxidation potential of protoplasm will be possible. Thanks to the work of CLARK, a beginning has already been made, as can be seen from the review of J. and D. M. NEEDHAM '26. But in the words of these authors, "Much investigation and much thought will be needed before the nature of the assumptions required in the application of oxidation-reduction potential to living systems is accurately understood". An important recent contribution is that of COHEN, CHAMBERS, and REZNIKOFF '28.

Oxidation-reduction reactions are not the only type of reactions which occur in protoplasm. Proteins are broken down or built up, fats are hydrolyzed, glycogen undergoes various transformations. Probably a cell contains at least twenty or thirty types of enzymes, all capable of furthering particular chemical reactions. It is difficult to understand how so many different reactions could go on more or less simultaneously within the narrow confines of a droplet of protoplasm. As a formal explanation of this difficulty, HOFMEISTER '01 proposed the theory that the cell was divided by partitions into many small chambers. Various enzymes were thus separated from each other, and each controlled its own type of reaction in its own allotted space, never diffusing into a neighboring compartment.

Although the theory of HOFMEISTER has been accepted by many, it certainly can not apply generally. As will be shown later (see chap. 5), some types of protoplasm have a very low viscosity, and this would be quite inconceivable if the cell were divided up by partitions. On the basis of our actual knowledge concerning the morphology and the physical properties of protoplasm, it is much more logical to assume that the chemical reactions of living cells occur most frequently at the surfaces of the protoplasmic granules. The conditions for chemical reaction are especially favorable at surfaces, and this is particularly true for enzyme reactions. The work of WARBURG (see for example WARBURG '21), offers definite evidence that certain reactions which occur in living systems are really surface reactions.

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## CHAPTER IV

### METHODS OF STUDY

Natural science depends on measurements. If the colloidal study of protoplasm is to prosper, methods must be developed which will enable the biologist to determine roughly, or if possible quantitatively, the colloidal properties of the living material. In a few cases the ordinary technique of the colloid chemist can be applied to the living cell, but in order to answer many important questions, one must employ methods specially adapted to the peculiar form and delicacy of protoplasm. Of the methods that have been used not all are good. Obviously no scientific method is without its limitations. One could hardly expect any great degree of accuracy in the physical determinations made on living cells. An inaccurate determination is certainly better than no determination at all. But it must be remembered that subjective impressions are more apt to hinder scientific progress than to advance it. And in its beginnings the colloid chemistry of protoplasm has had to suffer a great deal from the hastily published guesses of its pioneers.

There are three types of methods that have been used in the colloidal study of protoplasm: optical methods, methods of viscosity determination, and electrical methods. The classification is not an exact one. Indeed, one type of viscosity determination is almost purely an optical method.

In the last analysis, practically every colloidal investigation of the living cell involves optical study, for without a microscope the cell would typically be invisible. Mere examination with a microscope can give some information about the properties of the cell as a colloidal system. The sizes of the various granules in the protoplasm are important items which can be decided by direct microscopic study. Occasionally the appearance of new granules or the disappearance of some already present may be seen with the ordinary microscopic lenses. Sometimes new

films or membranes can be observed in process of formation. But as long as the biologist depended entirely on what he saw in the cell, his knowledge of its colloidal properties remained very slight. With a microscope alone, one can in general obtain only scant information regarding the physical properties of protoplasm. Nevertheless, in the past many authors have not hesitated to express bold and certain opinions on the basis of pure morphological observation. But what to one author looks like coagulation or gelation may to another seem like a solation. Protoplasm is an extremely intricate system, and frequently one is led astray by its complexities. Great care must be taken in interpreting observations. At least one writer has regarded the increase in volume of a cell as evidence of its solation or liquefaction. This may at first sight seem perfectly plausible, and yet frequently increase in volume of a cell is accompanied by a pronounced gelation.

It is quite possible that in some types of living material careful observation with an ordinary microscope may give very valuable information concerning colloid chemical processes. Thus in cells relatively free from granular material it might be possible to observe the formation of new granules or strands. It might also be possible to note gross changes in the refractive index of the protoplasm. But certainly in the past, purely morphological study of living cells has yielded very little definite and indisputable evidence concerning the colloidal properties of the living substance. This may have been due in part to the inability of the older cytologists to interpret their observations properly from a physical standpoint, but the main difficulty lies in the fact that physical changes in the protoplasm of a cell are frequently quite invisible with an ordinary microscope. Some cells may become completely coagulated or gelatinized without any obvious change in their appearance.

In recent years the colloid chemist has found good use for the polarization microscope. With it he has often been able to demonstrate shapes and arrangements of colloidal micellae. Fortunately the polarization microscope can also be used on biological material, and it frequently has been. Indeed long before there was a science of colloid chemistry, biologists were well acquainted with the polarization microscope and some of them contributed to the theory of its use. With the devel-

opment of staining and sectioning methods, interest in the polarization microscope waned, as biological workers became more and more involved in all the complicated technique of classical cytology. But even though stained and colored cells show many details which invite description, such cells are of course very much dead, and in recent years there has been a pronounced reaction against the type of cytology which makes no attempt to investigate the cell as a living object. New instruments for examining the living cell have been sought for. In late years, stimulated perhaps by the discoveries of the colloid chemists, biologists have again started to examine animal and plant material under crossed nicols. A book by W. J. SCHMIDT ('24b), gives a resumé of old and new work in this field. Most of the researches have been made on inanimate fibers, but some observers have examined living muscle and also other types of protoplasm.

The polarization microscope is a complicated instrument and its use could hardly be explained in a page or two. The recent book of AMBRONN and FREY '26 provides a fairly complete manual; it gives details both as to theory and practice. There is a pamphlet written by SCHMIDT '24a, which is simpler, but not so inclusive. In the main a polarization microscope is an ordinary microscope provided with one nicol prism below the object and one above. There is also provision for inserting thin plates of quartz, mica, etc., of various thicknesses and of various optical properties. When an object is viewed under crossed nicols, it appears black if it is isotropic, no matter in which position it lies on the stage of the microscope. Anisotropic or doubly refractive objects appear bright under crossed nicols, and as they are rotated on the stage of the microscope, there are two positions in which they appear brightest and two in which they appear darkest.

To be studied profitably under a polarization microscope, an object must be anisotropic. This anisotropy may depend on the presence of fibers or plates, or it may be due to the fact that the body is subjected to greater pressure in one plane than in another. The fact that fibrils show anisotropy explains why muscles and nerves are such excellent objects for study under the polarization microscope. Unfortunately undifferentiated protoplasm is typically isotropic (see p. 97), and it is thus not possible to study it with the polarization microscope.

In the development of the science of colloid chemistry, the ultramicroscope has played a very important part. This instrument makes possible the study of particles smaller than those which can be seen with the ordinary microscope. The principle of the ultramicroscope is quite simple. Ordinarily light is sent in parallel rays through the tube of the microscope. But in ultramicroscopic study the object is illuminated from the side and the image which is seen by the observer is not formed by the absorption of light but rather by the rays which are diffracted from the surface of the object.

Various textbooks of colloid chemistry and of microscopy give information regarding the theory of the ultramicroscope. Reference may also be made to the papers of SIEDENTOPF ('07 a, b; '08, '09). In ultramicroscopic study of protoplasm, either a paraboloid or a cardioid condenser is ordinarily employed.

In the use of the ultramicroscope for biological study, it must be remembered that the protoplasm is not free, but that it is surrounded by a membrane or membranes. This introduces one complication, and there are others. Apparently only certain material is favorable for study. The following quotation from PRICE '14 is instructive:

"Considerable difficulty is experienced in selecting material suitable for observation and experiments in various directions. GAIDUKOV showed that only in certain cases was good observation possible. The considerations affecting the suitability of material are roughly as follow: the tissues or structures must generally be only one cell in thickness; the walls of the cells must be optically homogeneous, the diameter of the cell or cell-filament must not be so small that the diffraction image of the walls interferes with observation of the cell contents; and the cells must not contain a large number of bodies of the nature of chloroplasts, as these, by scattering the light, interfere with good observation of the more minute structures."

Some authors in using the ultramicroscope have failed to recognize the limitations of the method when it is used on living material, and they have sometimes drawn too hasty conclusions regarding the presence or absence of colloidal particles, or the physical state of the protoplasm. When a cell is surrounded by a membrane or wall which appears bright in the darkfield, great caution must be exercised in interpreting results obtained from

a study of the interior. As an example of the inadequacy of the method under these conditions, it may be noted that in salamander blood cells, according to AGGAZZOTTI '10, the nuclei of the cells are almost or completely invisible when the cells are examined under darkfield. In AGGAZZOTTI's figures of these cells he shows no nuclei. In this case the light entering the field from the side is largely diffracted from the surface of the cell. Under conditions of this sort, the visibility of structures within the cell depends largely on the amount of light that enters. Changes in the physical structure of a cell wall or membrane may very readily have an effect on the amount of light that is able to enter the cell. In a recent paper, SHIWAGO '26 warns against interpretations arrived at by use of the darkfield, that is to say the ultramicroscope. Especially is this true in the study of the nucleus. Frequently, owing to the brightness of the nuclear surface, the interior of the nucleus appears optically empty in darkfield, although study under ordinary conditions of illumination clearly shows the presence of structures within it.

In studying the colloid chemistry of protoplasm, it is of great importance to be able to detect physical changes in the living substance. No property of colloidal systems is of more importance than the viscosity, and it is very fortunate that in recent years methods have been developed which make it possible to estimate and even to measure the viscosity of the protoplasm.

In most experiments, it is only necessary to determine the viscosity at a certain time or under a certain set of conditions. If, on the other hand, one wishes to compare the viscosity of protoplasm with the viscosity of inanimate fluids, it is essential to know the viscosity in absolute units. The measurement of the absolute viscosity of protoplasm is not a simple matter and the values that have been obtained have varied with the type of method employed. In the next chapter the whole subject of absolute viscosity determination will be examined critically, and for the present we shall consider only the measurement of relative viscosity.

When one crushes a cell under a cover slip, often the protoplasm emerges and flows into the surrounding medium. The speed of outflow depends on the viscosity of the protoplasm, and by observing cells subjected to pressure, several early observers were able to estimate protoplasmic viscosity (see REINKE '95,

ALBRECHT '98). Unfortunately when protoplasm emerges from a cell, it is no longer in a normal state. It may become mixed with the surrounding medium. Frequently as the protoplasm starts to emerge from the cell, it becomes surrounded by a film or membrane (see chap. 13). The nature of this film or membrane varies under different conditions. Under certain conditions it forms much more rapidly than under others. There are thus two factors which govern the speed of outflow of protoplasm from a cell. One is the viscosity of the protoplasm, the other is the reaction which the protoplasm undergoes when it comes in contact with the outer medium. This second factor can be very important. Thus, if one crushes *Arbacia* eggs in sea-water and in isotonic sodium chloride solution, the speed of emergence of the protoplasm is much greater in the second case. This is due not to a lower viscosity, but to the fact that in sea-water *Arbacia* egg protoplasm forms a film or membrane as it emerges, and no such film forms in isotonic sodium chloride solution in the absence of calcium (see p. 222).

In recent years, many workers have studied the viscosity of protoplasm by inserting fine glass needles into living cells. This microdissection method was introduced by KITE '13. He employed a Barber pipette holder for the mechanical manipulation of the needles. Following KITE, more perfect apparatus has been developed, and microdissection instruments are now to be found in most biological laboratories. The two most common types are the CHAMBERS microdissection apparatus, and the PÉTERFI micromanipulator. The former is sold by LEITZ, the latter by ZEISS. Other types of apparatus have also been described (BISHOP and THARALDSEN '21, TAYLOR '25c). For a general discussion of method, see CHAMBERS '22, PÉTERFI '24, '27b. When a cell is to be dissected, it is placed in a small hanging droplet of fluid in a moist chamber and is there operated upon.

Beyond any question, the microdissection and microinjection methods have found many uses in biology and will doubtless continue to find many more. For the measurement of viscosity however, the microdissection method can at best give only indications of gross differences in viscosity. And even for these it is more or less uncertain. Microdissectionists themselves now generally believe that the introduction of a needle or a pipette into a cell produces injury (see for example PÉTERFI '27,



CHAMBERS and POLLACK '27, HOWLAND and POLLACK '27). Such injury might conceivably cause either increase or decrease of viscosity. It is sometimes a little difficult to understand just what criteria are employed for viscosity determination by the users of the microdissection method. In some instances the experimenter seems to rely most on his general impressions, as he watches the needle pass through a cell. In many cases, apparently, the viscosity is judged largely from the speed of flow of protoplasm from injured cells. Thus, for example, SEIFRIZ '26b states: "Owing to the extraordinary thinness of the cytoplasmic layer lining the wall of the amphibian red cell it is quite impossible to obtain any direct evidence of the consistency of the protoplasm. The cytoplasm can not be made to flow out of the corpuscle and retain its identity, as is possible in other types of cells, and thus permit a determination of the consistency with a fair degree of accuracy." For some microdissectionists, the fact that certain types of protoplasm can be cut into discrete fragments is taken as evidence of high viscosity. If the microdissectionist makes his viscosity determinations on the basis of the speed of outflow of protoplasm from a cell, or on the basis of the fact that some cells can be cut into fragments and others not, then such determinations are subject to the same objections already considered for the case in which the protoplasm is squeezed out of the cells. Here too, just as in the case of the cells crushed under a cover slip, the reaction between the naked protoplasm and the surrounding medium certainly plays an important rôle. Consider for example the case previously mentioned of *Arbacia* eggs in isotonic sodium chloride solution and in sea-water. In the sea-water the eggs can be cut into discrete fragments, whereas in the isotonic sodium chloride solution in the absence of calcium, the protoplasm disperses as soon as the cell is cut. To a microdissectionist this would mean that the protoplasm was more viscous in sea-water, which is quite the opposite of the truth (see chap. 9). The fact that the cells can be cut into discrete fragments in the presence of calcium, and not otherwise, is due to the fact that the formation of a film or membrane around the naked protoplasm depends on the presence of calcium (see chap. 13).

In 1920, SEIFRIZ made an attempt to put the microdissection method of measuring viscosity on a semi-quantitative basis. He arranged a series of ten "viscosity values" which he based

on the viscosity of various known substances. These are shown in the following table, which is taken from SEIFRIZ's paper.

Scale of viscosity values according to SEIFRIZ

Viscosity value	Descriptive term	Percentage of gelatine	Substances having an equivalent viscosity
1	Watery . . . . .	0.0	Water
2	Very liquid . . . . .	0.05	—
3	Liquid . . . . .	0.2	—
4	Slightly viscous . . . . .	0.4	—
5	Rather viscous . . . . .	0.5	Paraffine oil
6	Decidedly viscous . . . . .	0.6	—
7	Very viscous . . . . .	0.7	Glycerine
8	Extremely viscous . . . . .	0.8	Bread-dough
9	Gel . . . . .	1.0	Vaseline
10	Rigid gel . . . . .	2.0	Firm gelatine

Regarding the gelatin he used in his comparison values, SEIFRIZ says that it formed a soft jelly at 18° C, when the concentration was as low as 0,8 %. Presumably in concentrations lower than this the gelatin remained fluid.

In a recent paper (HEILBRUNN '27a), the following statement was made: "That anyone should be able to measure viscosity accurately by observing the passage of a mechanically controlled needle through protoplasm seems on the face of it unlikely. It would be easy to put the matter to a test. If a microdissectionist were given certain unknown samples of fluids, it would be interesting to see how his estimates of their viscosity compared with the known values."

When the above sentences were written it was not realized that the table of SEIFRIZ was an actual example of such a test. Solutions of gelatin so dilute that they do not gel have a viscosity only about twice that of water. True, the viscosity varies at different rates of shear (see for example FREUNDLICH and ABRAMSON '27), and the rate of shear in SEIFRIZ's needle tests is not defined. But even at very low rates of shear the viscosity of dilute gelatin sols is probably not over three or four times that of water. And yet SEIFRIZ regards the viscosity of a 0,7 % gelatin sol as equal to that of glycerine, which has a viscosity approximately

a thousand times that of water. A method which even occasionally permits of an error of 20 000 % can scarcely be regarded as a reliable method.

As early as 1880, it was known that the starch grains of plant cells moved under the influence of gravity and became accumulated in the lower part of the cell (see DEHNECKE '80, HEINE '85). NĚMEC '01a, noticed that the speed of fall of such grains was much slower at a temperature of 6° C., and he suggested that this was due to an increase in the viscosity of the protoplasm. HEILBRONN '12, '14, first proposed to use the speed of fall of starch grains as a measure of the protoplasmic viscosity. As a matter of fact, the speed of fall of spheres through fluids is frequently used by physicists and physical chemists as a measure of the viscosity, and not a few viscosimeters have been constructed for the purpose of determining viscosity of inanimate fluids in this manner. The principle governing all these measurements finds mathematical expression in the well known equation of Stokes. This equation will be considered in the next chapter, for in the determination of absolute viscosity certain corrections must be applied to it. These corrections are much less important for the measurement of relative viscosity, and for the present we will assume, as HEILBRONN does, that the viscosity is strictly proportional to the time of fall of particles through a fluid. In order to measure the time of fall, HEILBRONN cut longitudinal sections through young seedlings and placed them on the stage of a microscope tilted in such a fashion that the stage was vertical. The sections were cut in such a way that at least one layer of cells remained intact. Care had to be taken to study none but intact cells. Even in these the protoplasm showed effects of the sectioning process. Immediately after sectioning, the viscosity was high, due to the stimulation of the wound. After 10 or 15 minutes the viscosity decreased rapidly, remaining low for perhaps three hours, after which there was a pronounced viscosity increase, which HEILBRONN interpreted as due to the death of the cells.

Beyond any question the gravity method of measuring protoplasmic viscosity is a very valuable one. It must nevertheless be admitted that the cells in thin sections of plant tissues are far from being in a normal state. The very fact that they die within three hours is an indication that they are not in the same condition that they were in before sectioning. Moreover the

effect of wounding may be a serious complicating factor (compare ZOLLIKOFER '18). In the use of the gravity method, it would seem far better practice to expose the intact plants or tissues to the action of gravity, and then to cut sections later. In this way any effects of wounding would be avoided. As a matter of fact NĚMEC as early as 1901 made a few viscosity tests in this way. Recently BURNING '26a has also used the gravity method on intact masses of tissue.

Certain types of plant material are more favorable for study by the gravity method than are others. In the opinion of Prof. WEBER, who has used the gravity method more than any other worker, starch sheath cells are not as favorable as cells richer in protoplasm, for example root cap cells, or cells of the coleoptil of wheat seedlings (personal communication).

The granules and inclusions of animal cells do not ordinarily move under the influence of gravity, and the movement when it occurs is usually very slow. It is advantageous therefore to subject these cells to a stronger force than that of gravity. With a small hand centrifuge or with an electric centrifuge, a centrifugal force can be developed which may be thousands of times as great as gravity. Fortunately living cells can be subjected to centrifugal force without injury. Many workers in the field of experimental embryology have used the centrifuge in the attempt to analyze the developmental potencies of various visible structures of marine ova (for literature see MORGAN '27). In these experiments, granules were shifted to one side or the other of the egg, so that, later, definite regions of the embryo contained certain granules and not others. In most instances, in spite of the abnormal distribution of the granular material, the embryos developed perfectly normally even after prolonged and drastic centrifugal treatment.

The embryologists who centrifuged eggs, frequently noted that the granules moved more readily at one stage than at another. Generally they referred these differences to differences in the viscosity of the protoplasm, but they made no systematic observations on the viscosity differences, and their published statements consist of a few scattered remarks. Only in relatively recent years has it been realized that the centrifuge method is an extremely important means of determining the viscosity of living protoplasm.

As has already been mentioned, the speed with which a particle moves through a fluid varies inversely with the viscosity. For relative measurements of viscosity it is only necessary to determine the speed with which the protoplasmic granules move under the influence of a given centrifugal force. In this way, for a particular type of protoplasm, one can discover how the viscosity changes from time to time, or how it changes when the protoplasm is treated with various reagents. In the next chapter, it will be shown that absolute viscosity can also be determined by the centrifuge method. Some further details regarding the theory of viscosity measurement by the centrifuge method will then be considered. The use of the centrifuge for viscosity

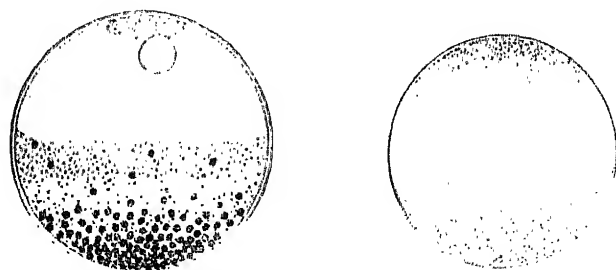


Fig. 1. A. Centrifuged egg of *Arbacia*, after LYON '07 and MORGAN and SPOONER '09. B. Centrifuged egg of *Cumingia*, after MORGAN '10.

determination requires no technical skill. Measurements can be made in a relatively short time. If one only wishes to know whether the viscosity is increased or decreased by a given reagent, it is merely necessary to centrifuge treated and untreated cells simultaneously (in separate tubes) for an appropriate time, and then to note the degree of granular movement under the microscope. On the other hand, if one wishes a more exact measure of the change in viscosity produced by the reagent, it is necessary to make a series of tests in which the cells are centrifuged for varying intervals of time. In this way it is possible to determine how many seconds are required to move the granules a certain distance through the cell, and this gives the speed. A few practical hints with regard to the centrifuge method of measuring protoplasmic viscosity are given in a recent paper (HEILBRUNN '26a).

In order to obtain an idea of what happens when cells are centrifuged, reference may be made to Fig. 1. This shows a centrifuged egg of the sea-urchin *Arbacia* and a centrifuged egg of the clam *Cumingia*.

The use of the centrifuge method is not limited to animal cells. Following some older experiments in which the centrifuge was used for other purposes (DEHNECKE '80, MOTTLER '99, ANDREWS '03), in recent years various observers have determined viscosity in plant cells with the aid of centrifugal force. The first worker to employ the centrifuge for the measurement of the protoplasmic viscosity of plant cells was SZÜCS '13.

One advantage of the centrifuge method lies in the fact that it can be used on relatively large masses of material. Whole animals can be centrifuged, and their tissues can then be fixed and sectioned in order to determine the speed of granular movement in the individual cells. In such tests rapid fixation is essential, for the granules following centrifugal treatment slowly return to their original positions. It is doubtful if results can ever be obtained with small cells, for in such cells the proximity of the cell wall exerts a retarding influence on the movement of the granules, and so does the nuclear membrane, if the nucleus is of relatively large size. In large nuclei it is sometimes possible to observe movements of nucleoli or chromatin under the influence of centrifugal force, so that it might be possible to determine the viscosity of the interior of the nucleus. Until now, no such determinations have been made.

In using the centrifuge method to measure relative viscosity, it must be remembered that the speed with which the granules move depends not only on the viscosity, but on various other factors as well. Were the size of the granules to change, or their specific gravity, this would make a difference in their rate of travel. Various reagents might very well alter the size or the specific gravity of protoplasmic granules, and the observer should be on his guard against the possibility of such an effect. Changes in size, if appreciable, ought to be visible under the microscope. In those instances in which cells contain both heavy and light granules which move to opposite ends of the cell on being centrifuged, the possibility of error due to changes in the specific gravity of granules can usually be excluded. For if both types of granules show the same variations in speed under different

conditions, one can be quite certain that the differences are due to viscosity and not to changes in specific gravity. Suppose for instance that a reagent caused an increase in the specific gravity of the heavier granules. It would be quite improbable that the same reagent would cause an exactly comparable decrease in the specific gravity of the lighter granules, and yet unless such an equivalent decrease occurred, the movement of the lighter granules would be relatively slower.

To some authors (e. g. CHAMBERS '21, '24 first imprint but not second), it has seemed that centrifugal treatment of cells might very well alter the viscosity, that the movement of granules through the protoplasm might tend to injure or to liquefy it. An effect of this sort would not have as much importance for measurements of relative viscosity. If a given sort of protoplasm were centrifuged at two times or under different conditions, if it were more viscous at one time than at another, it would offer more resistance to the movement of granules even if this movement did gradually cause liquefaction. As a matter of fact, the only actual tests that have been made indicate that the movement of granules through the protoplasm has no liquefying action at all. It is certain that if such a liquefying action occurred, or if the centrifugal treatment broke down fibers or a network in the protoplasm, then centrifuge tests at a higher rate of speed should give lower viscosity values. For if the shearing force of the granules is greater, then the viscosity should be less, on the assumption that the movement of the granules produces a lowering of viscosity. But, as a matter of fact, tests both with *Arbacia* and *Cumingia* eggs, show that no matter how fast or how slow the centrifuge is turned, the same viscosity values are arrived at. The data for the *Cumingia* egg will be presented in a later chapter (see p. 94).

The use of the centrifuge method for the cells of the alga *Spirogyra* presents a special case, and in view of the fact that these cells have often been used in viscosity measurements, a few words regarding them will not be out of place. *Spirogyra* cells, as everybody knows, contain large spiral chromatophores. It is the speed of displacement of these chromatophores that is used as an index of protoplasmic viscosity. The physical structure of the *Spirogyra* cell is not well understood, but we can with justice assume that the chromatophore lies loosely imbedded

in a layer of protoplasm which completely lines the cell wall. Under the influence of centrifugal force, the chromatophore tends to move past the cell wall, from which it is separated by a layer of fluid. Viscosity is defined as the resistance offered by a fluid lying between two planes to the movement of one plane over the other\*. Obviously the speed of movement of the chromatophore in the *Spirogyra* cell is an inverse measure of the viscosity of the protoplasm in which it is imbedded. If the measurement is to be exact, it is of course essential that the chromatophore does not itself undergo a marked change in physical state.

A very useful method for the measurement of protoplasmic viscosity is the determination of the speed of Brownian movement of granules in the cytoplasm. In comparatively recent years, this method has been used by CHIFFLOT and GAUTIER '05, by \* LEBLOND '19a, b, by BAYLISS '20, and others, and there is apparently a general belief that the use of Brownian movement to measure viscosity is a twentieth century development. As a matter of fact, soon after the middle of the nineteenth century many biologists made observations on Brownian movement in living cells, and at least some of these workers regarded their observations as a source of information regarding the fluidity or viscosity of the protoplasm. Thus LIEBERKÜHN in 1870 begins a long paper with the following sentence: "In den nachfolgenden Mitteilungen wird vielfach die Brownsche Molekularbewegung benutzt, um über die Anwesenheit leichtflüssiger Substanzen zu entscheiden."

In general, the observations that have been made on Brownian movement are not very exact. The movement is seen to be vigorous at one time, and less vigorous or absent at another. There have been no published papers on the actual speed of the granules, and measurements of such speed would usually be very difficult to make. However, in some cells it is possible to follow the movements of a single granule and in this way to determine the speed. In other cells the granules are so crowded together that

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\* More exactly, according to Maxwell: The viscosity of a substance is measured by the tangential force on unit area of either of two horizontal planes at unit distance apart, one of which is fixed while the other moves with the unit of velocity, the space between being filled with the viscous substance.



no observation of their Brownian movement is possible. This is often the case in marine eggs. In such instances the speed of Brownian movement may sometimes be determined by estimating the speed with which the granules return through the cell, after they have been centrifuged into one end. An example of such a determination will be given in the next chapter.

In 1922, HEILBRONN described a new method of viscosity measurement, the magnetic method. Small iron particles were introduced into the protoplasm of slime molds, and the protoplasm was then placed in the magnetic field of an electro-magnet. In his first experiments, HEILBRONN determined the distance from the magnet and hence the magnetic force necessary to cause a barely perceptible movement of a small iron sphere. Later, he studied the twisting of iron rods within the protoplasm. As HEILBRONN himself points out, these methods are only possible for large masses of protoplasm. Details regarding HEILBRONN's measurements will be found in the next chapter (see p. 62). SEIFRIZ '24 modified HEILBRONN's method. He used nickel particles, and injected them with the aid of a micropipette. In order to measure viscosity, SEIFRIZ determined the speed with which the nickel particle approached the electromagnet. For a further discussion of SEIFRIZ's experiments, see p. 64.

Still another method of viscosity determination is the plasmolysis form method recently advocated by WEBER (see WEBER '21 a, '24 a, c, d; '25 a, b, c, d). When plant cells are plasmolyzed, the border of the cell may be perfectly smooth, in which case the plasmolysis form is said to be "convex", or it may be angular ("eckig"). When the border of the cell is extremely uneven, the plasmolysis is said to be a "Krampfplasmolyse". Sometimes the term "concave" is used as opposed to convex. For illustrations of various types of plasmolysis form, see Fig. 2. The reason for the different types of plasmolysis under different conditions was at first uncertain. It seemed quite probable that surface conditions might play an important rôle. However, WEBER has shown that in general there is a perfect correlation between the type of plasmolysis and the viscosity of the interior of the protoplasm as determined by the centrifuge method. With low protoplasmic viscosity, convex plasmolysis occurs. As the viscosity increases, the plasmolysis becomes angular or "eckig", and with still higher viscosity, "Krampfplasmolyse" appears. It seems

fairly safe, therefore, to use the plasmolysis form as an indicator of the relative viscosity of the interior protoplasm. The plasmolysis

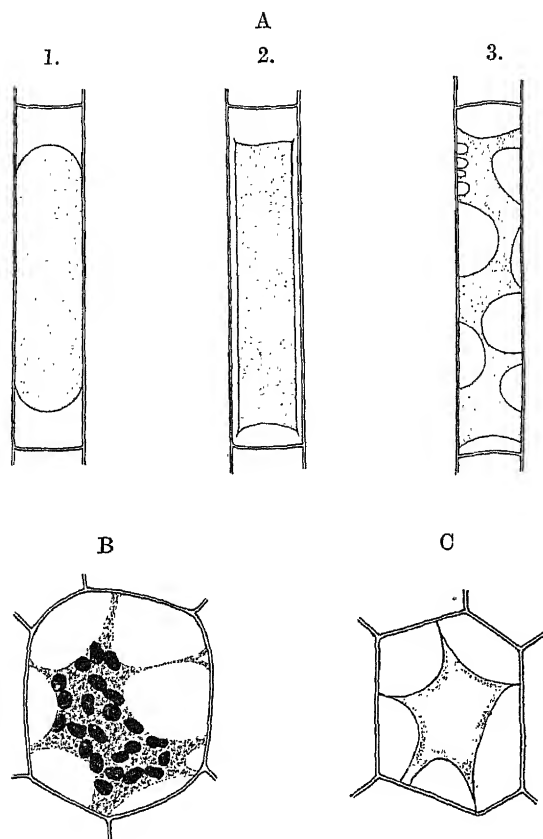


Fig. 2. Diagrams of plasmolysis form. A: *Spirogyra*, semidiagrammatic, chloroplast not shown; 1. convex, 2. "eekig", 3. concave ("Krampfplasmolyse"). B. *Epiphyllum*, concave plasmolysis, after PRINGSHEIM '54. C. *Dentaria*, concave plasmolysis, after NÄGELI '55. These diagrams were made under the direction of Prof. WEBER.

form method of estimating protoplasmic viscosity has the advantage that it is very simple and that it can be employed on some types of material that are not easily tested by other methods.

In many plant cells the protoplasm is more or less continually in a state of flow. Botanists have frequently measured the rate of such flow under various conditions, and the literature of plant physiology contains numerous valuable observations of this sort. It is beyond any question logical to assume that the speed of protoplasmic streaming decreases with increasing viscosity. But if, under a given set of conditions, it is found that the rate of protoplasmic streaming decreases, one can never be certain that the effect is due to an influence on the viscosity or to a direct influence on the force or forces which occasion the protoplasmic streaming. Until these forces are known, we can only regard the data on protoplasmic streaming as suggestive. In this connection it is interesting to note that HEILBRONN '14 measured both the rate of protoplasmic streaming and the viscosity of the protoplasm in the cells of longitudinal sections of the stem of *Vicia faba* seedlings. In such cells, the viscosity decreased for two hours and twenty minutes and then underwent a sharp increase. For an hour and a half, the speed of protoplasmic streaming increased with the decrease in viscosity and at a corresponding rate, then however the speed of protoplasmic streaming began to decrease even though the viscosity of the protoplasm was still decreasing. It is thus obvious that the speed of protoplasmic streaming is not always a perfect measure of the viscosity.

BĚLEHRÁDEK '24-'25 has attempted to obtain a more accurate measure of the viscosity from the speed of protoplasmic streaming. It has long been known that in the moving protoplasm of a plant cell, the tiny granules move at a faster rate than the chloroplasts. BĚLEHRÁDEK believes that the ratio of the speed of the chloroplasts to that of the tiny granules is a measure of the viscosity. In support of this rather unusual idea, BĚLEHRÁDEK states that with increasing viscosity the protoplasm would tend to pull along the chloroplasts and would more and more prevent them from being retarded by the cell wall. In the standard definition of viscosity, we conceive of two parallel planes, each of unit area, as being of unit distance from each other and separated by a given substance. The retardation which one plane suffers in moving over the other is a measure of the viscosity. Obviously from this definition, with increasing viscosity of the protoplasmic fluid between cell wall and chloroplast, and between cell wall and granule, there will be an increasing retardation of

the speed of the chloroplast or granule, and this retardation will be greater the greater the surface of the chloroplast or granule. This is evidently a very important factor, but it has been completely neglected by BĚLEHRÁDEK, who seems to think that the greater the viscosity the greater the speed of the chloroplast. As a matter of fact, BĚLEHRÁDEK himself states that it is almost impossible to determine in any rigorous manner in what manner the viscosity of the protoplasm depends on the ratio of chloroplast speed to granule speed. Not being able to solve the problem, BĚLEHRÁDEK is content simply to divide chloroplast speed by granule speed and to regard this simple fraction as a measure of the viscosity. From what has gone before, it appears somewhat doubtful if it would not be better to divide granule speed by chloroplast speed instead of chloroplast speed by granule speed.

Even more unconvincing is the method of viscosity determination proposed by BĚLEHRÁDEK in 1926. By measuring the rate of biological processes at two different temperatures, he hopes to estimate the viscosity of the protoplasm on the assumption that the speed of the process is governed by one chemical reaction which obeys the formula he proposes. The various complications involved in the interpretation of temperature coefficients have to some extent been outlined in a short note, HELBRUNN '25e. To ignore all these complicating factors, to assume one reaction instead of many, to assume moreover that colloidal viscosity acts in the same fashion as the viscosity of true solutions on the rate of chemical reaction, all these assumptions may lead to a formulation which permits of viscosity measurement; but for the present at least, until the nature of the assumptions and their justification is more clearly understood, it seems probable that the more direct types of viscosity determination are to be preferred.

The electrical properties of colloids are very important. When an electric current is sent through a colloidal solution, the colloidal particles typically wander either to one electrode or the other. This is the well known phenomenon of cataphoresis. Many investigators have studied the cataphoresis of living cells. This can very conveniently be followed, or rather measured, under the microscope. For the technique of such measurement, see MICHAELIS '21. Special apparatus has also been devised by NORTROP and KUNITZ (see NORTROP '22, KUNITZ '23, NOR-

THROP and KUNITZ '25). In this type of apparatus, cells can be observed to migrate in a closed chamber bounded by parallel walls. The cataphoretic velocity is measured at a point halfway between the bottom and top of the chamber, and also at a point one-sixth of the distance between bottom and top, or these values can be obtained from a curve in which the cataphoretic velocity is plotted against various arbitrary distances from top or bottom (see FREUNDLICH and ABRAMSON '27). The true cataphoretic velocity can then be calculated from the formula of VON SMOLUCHOWSKI:

$$w = \frac{3}{4}w_{\frac{1}{2}} + \frac{1}{4}w_{\frac{1}{6}},$$

in which  $w$  is the true cataphoretic velocity,  $w_{\frac{1}{2}}$  the cataphoretic velocity in the center of the chamber, and  $w_{\frac{1}{6}}$  the cataphoretic velocity at a point one-sixth of the distance between bottom and top.

In studying the electric properties of protoplasm, it is advantageous to have electrodes which can be introduced directly into the cell. The problem of constructing non-polarizable electrodes which could serve this purpose was not a simple one. It has however been successfully met by ETTISCH and PÉTERFI '25, and by various other workers (TAYLOR '25, GELFAN '26, BUYTENDIJK and WOERDEMAN '27). The technical details of all these types of microelectrodes can not be considered here. Their construction involves no new theoretical development, and they represent modifications of various standard electrodes in which a long and very fine capillary pipette is attached to the main body of the electrode. The pipette is of the type used in microdissection, and generally has a diameter of only 1—2 microns. In the ETTISCH-PÉTERFI, TAYLOR and GELFAN instruments, it is filled with agar. Because of their small bore, the microelectrodes have a very great electrical resistance. They are attached to an ordinary microdissection apparatus, and are moved about in the same way that microdissection needles are.

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## CHAPTER V

# THE ABSOLUTE VISCOSITY OF PROTOPLASM

Microscopical study has shown that protoplasm is typically a concentrated suspension of visible granules. Are these granules suspended in a relatively viscous medium or in a fluid medium? Some authors would have us believe that the intergranular material of protoplasm is normally a gel of high viscosity. Others hold that protoplasm is relatively fluid and that the intergranular material must necessarily be fluid.

As a matter of fact, this difference in opinion is by no means a new development. For many years biologists have argued as to whether the living substance is fluid or solid. The question is in part a question of definition. We can define a fluid as without form, or amorphous, flowing readily, and so adapting itself to the shape of the vessel which contains it. If we have a gelatin jelly in a test tube and invert it, the gelatin does not flow out when the tube is inverted. It is a gel. But if we now subject the gelatin to heat, it flows out of the tube readily. It has become a fluid sol. For the colloid chemist, this is the usual conception of sol or gel. Actually the distinction we have made is rather an artificial one. With a certain size of test tube, and under the influence of a certain force, i. e. gravity, the gel does not flow. But if we apply a greater force, it does flow. Our distinction breaks down. Theoretically it is better to follow MAXWELL and to define the fluid state as a state in which flow occurs even when the force exerted upon the substance is infinitely small. For a true fluid, the rate of flow is proportional to the shearing force, that is to say the force tending to produce distortion or movement. When the rate of flow is not proportional to the shearing force, a substance is elastic and can no longer be considered as a true fluid. For some authors it is an elastic fluid, others prefer to call it a plastic solid.

For the present, such absolute distinctions between solid and fluid states need not concern us. In the next chapter, we will consider the question of elasticity. The problem to be discussed at this point is whether or not protoplasm is highly viscous. Is it sol or gel in the ordinary sense in which these terms are used? Actually the problem is a twofold one. Inasmuch as protoplasm is a suspension, we can consider either the viscosity of the suspension as a whole, or the viscosity of the dispersion medium, that is to say the intergranular material. A watery suspension can be so concentrated that its viscosity may reach a value many times that of water. As the concentration is increased, the viscosity may eventually become infinite. It is possible to determine both the viscosity of the entire protoplasm and the viscosity of the intergranular material.

The earliest measurements of absolute protoplasmic viscosity were made by the German botanist HEILBRONN in 1914. In the starch sheath cells of *Vicia faba*, a leguminous plant, the starch grains can be observed to fall under the influence of gravity. By measuring the speed with which the grains fall, and comparing it with the speed of fall in water, HEILBRONN was able to determine the absolute viscosity of the protoplasm of these cells, on the assumption that the speed of fall was inversely proportional to the viscosity in the two cases. This assumption is on the whole justifiable, but it is not strictly true.

The law governing the speed of movement of a spherical particle through a fluid was first stated by STOKES in 1850. According to this law,

$$V = \frac{2g(\sigma - \rho)a^2}{9\eta}$$

in which  $V$  is the velocity,  $g$  the gravity constant,  $a$  the radius,  $\sigma$  the specific gravity of the particle,  $\rho$  that of the medium, and  $\eta$  the viscosity. From this formula it is obvious that if  $g$ ,  $\sigma$ ,  $\rho$ , and  $a$  remain constant, then the viscosity is inversely proportional to the velocity of the particle or directly proportional to the time of fall if the particle is moving under the influence of gravity. It can safely be assumed that when one measures the speed of drop of starch grains inside a cell and outside, that the diameter of the starch grain will not change appreciably. Its specific gravity will also presumably remain constant, and of course  $g$

will not change. But it must be remembered that the specific gravity of the protoplasm is different from that of water. It is somewhat greater. In a liquid of greater specific gravity, the starch grains would tend to drop more slowly. The specific gravity of the protoplasm of the *Vicia faba* cells is not known. Other types of protoplasm have a specific gravity of about 1.05 to 1.10. If one estimates the absolute viscosity of protoplasm by comparing the speed of drop of starch grains in water and in the cell, one should perhaps make allowance for the difference in specific gravity of the two fluids. However if one assumes that the specific gravity of protoplasm is no greater than that of water, the error is not large. Probably the error in HEILBRONN's measurements from this factor is roughly about 10 percent. That is to say, his values are 10 percent too high. Such an error could certainly be disregarded, except for the fact that there are other corrections which must also be made to HEILBRONN's values.

In order to understand these corrections, we must discuss the applicability of STOKES' law. And in view of the fact that later in the chapter it will be necessary to refer to STOKES' law again, the discussion will be made fairly complete.

The theoretical derivation of STOKES' law involves a number of assumptions. ARNOLD\* lists five of them as follows:

1. That the discontinuities of the fluid are small compared with the size of the sphere.
  2. That the fluid is infinite in extent.
  3. That the sphere is smooth and rigid.
  4. That there is no slip at the surface between sphere and fluid.
  5. That the velocity of the sphere is small.
- To these might be added:
6. That the particle is truly spherical.
  7. That the particle is a single particle and not one of many.
  8. That electrical forces may be disregarded.

The first assumption is chiefly important for very tiny spheres falling through gases. For liquids it may generally be disregarded. The second assumption was investigated theoretic-

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\* ARNOLD 1911, Phil. Mag., ser. 6, vol. 22, p. 755:



cally and experimentally by LADENBURG\*, who introduced the following form of STOKES' law for the case of a sphere falling through a fluid contained in a cylinder:

$$V = \frac{2g(\sigma - \rho)a^2}{9\left(1 + 2.4\frac{a}{R}\right)\left(1 + 3.1\frac{a}{L}\right)\eta}$$

In this formula,  $R$  is the radius of the containing cylinder,  $L$  is its length, the other symbols are as before. If we are to apply LADENBURG's correction to the cells of *Vicia faba*, we must know the diameter of the starch grains and the length and width of the starch sheath cells, or rather the length and width of the layer of protoplasm through which the starch grains fall. Certainly within a cell the speed of fall of a starch grain would be decidedly less than outside of a cell. If LADENBURG's correction is neglected, the values obtained for the absolute viscosity are too high, and perhaps as much as 20 or 30 percent too high, or even more.

The third and fourth assumptions are probably not very important in the application of STOKES' law to protoplasm. Most protoplasmic inclusions are smooth in so far as they can be seen by microscopic observation, and in general they appear to be rigid. We are also safe in assuming that the slip between a particle enclosed in protoplasm and the protoplasm itself is zero or approximately zero. Since the time of POISEUILLE, it has generally been supposed that when a substance is wetted by water, its coefficient of slip in relation to water is zero. Evidence for this view is given by WHETHAM\*\*, who found that there was no measurable slip when water flowed over various metals, or even over a film of oil.

In the derivation of STOKES' law, it is assumed that the velocity of the moving sphere or particle is so small that higher powers of the velocity may be neglected. For small particles falling under the influence of gravity, this is certainly true.

In the derivation of STOKES' law, it is assumed that the moving body is spherical. Most of the particles found in protoplasm are spherical. Starch grains are often not perfect spheres, but they

\* LADENBURG 1907, Ann. d. Physik, ser. 4, vol. 23, p. 447.

\*\* WHETHAM 1890, Proc. Roy. Soc., vol. 48, p. 225.

are nearly enough spherical so that STOKES' law probably applies to them. Moreover, in a comparison of the fall rate of the same particles in different fluids, any factor due to the non-spherical shape would probably cancel out.

STOKES' law was derived for the movement of a single sphere through a fluid and not for the movement of many. When many spheres move together, the fluid between them must of course move in the opposite direction. The presence of many spheres interferes with such a return flow. The nearer the spheres are to each other, the greater the resistance to this return flow, and the more slowly do the spheres move under the application of a given force. Thus, to return to the case under consideration, in a liquid of given viscosity many starch grains falling together would fall much less rapidly than a single starch grain falling alone. CUNNINGHAM\* has attempted to calculate a correction for STOKES' law which would apply to the case of many particles moving through a fluid. According to CUNNINGHAM,

$$V = \frac{2g(\sigma - \rho)a^2}{9\eta\gamma}$$

in which

$$\gamma = \frac{4b(b^5 - a^5)}{(b-a)^2(4b^4 - b^3a - 6b^2a^2 - ba^3 - 4a^4)}.$$

In this expression,  $a$  is again the radius of the particle,  $b$  is half the distance between the centers of two adjacent particles. But in making the computation, CUNNINGHAM prefers to substitute

for  $b$ , a quantity  $b'$  which is equal to  $b\sqrt{\frac{3}{2}}$ . The correction

of CUNNINGHAM is admittedly only a rough approximation to the truth. One can be certain, however, that when many particles fall through a fluid, the speed of drop of any one particle is greatly retarded. CUNNINGHAM gives the following calculated values of  $q$  for various values of  $b$  and  $b'$ :

$b'/a$	2	3	4	5	6	7	8	9	10	20	30	$\infty$
$b/a$	1.63	2.45	3.26	4.08	4.90	5.71	6.50	7.35	8.16	16.3	24.5	$\infty$
$q$	7.29	3.02	2.10	1.76	1.57	1.46	1.38	1.32	1.28	1.14	1.08	1

\* CUNNINGHAM 1910, Proc. Roy. Soc., ser. A, vol. 83, p. 357.

Finally it should be pointed out that STOKES' law does not consider a possible electric charge on the moving sphere. Practically all small particles suspended in water do have an electric charge, and such a charge would tend to retard their movement. But the effect of an electric charge on the speed of movement only becomes important when the particles are of submicron or amicon size, that is to say, when they are so small as to be invisible with the highest powers of the microscope\*.

Let us now return to the case of the *Vicia* cells studied by HEILBRONN. It will be remembered that he determined the time it took starch grains within the cell to drop a certain distance, and then compared it with the time of drop of similar grains outside the cell. The ratio between the two times was taken as the viscosity of the protoplasm of the cell. From the discussion of STOKES' law, it is obvious that starch grains within a living cell would tend to drop more slowly, not only because of the greater viscosity of the protoplasm, but also because of three other factors. These three factors are, first the fact that protoplasm has a greater specific gravity than water, second the presence of cell walls which would tend to retard the fall of the starch grains, and third the fact that the starch grains within the cell are numerous and in fairly close proximity to each other. The first of these factors is relatively unimportant, the second might make a difference of twenty or thirty percent at most, whereas the third factor might produce a very great effect. All of them would tend to make the viscosity values of HEILBRONN for *Vicia* cells too high. In addition we should perhaps also consider the fact that HEILBRONN studied cells in thin sections. Under these conditions there is an injury effect which may produce viscosity increase (see Chap. 8).

What HEILBRONN actually found was that the time of fall of the starch grains within the cell was in some cases only 8 times as great as the time of fall outside the cell. In other cells the ratio was somewhat greater, and he occasionally found cells in which the starch grains did not fall at all, and in which he assumed the viscosity to be infinity. These cells with infinite viscosity were apparently in a pathological state, and were probably dead.

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\* See VON SMOLUCHOWSKI, 1916, *Kolloid-Zeitschr.*, vol. 18, p. 190.

The interesting thing about HEILBRONN's measurements is that he showed for the first time that protoplasm in some cases at least was not the extremely viscous material that many authors had supposed. Books on cytology often speak of protoplasm as a very viscous fluid and the student of elementary biology is always impressed with the high viscosity of the living substance. The early writers on protoplasm often referred to it as a slime, that is to say a fluid of very high viscosity. Of course, after all, to say that a fluid is very viscous means but little, it may be very viscous in comparison with ether, and at the same time very fluid in comparison with a heavy oil. For purposes of orientation, one may consult the following table of viscosity values, compiled for the most part from the physical tables of LANDOLT-BÖRNSTEIN.

Viscosity of various fluids at 20° C. in absolute units

Acetaldehyde . . . . .	0.002
Chloroform . . . . .	0.0057
Benzol . . . . .	0.0065
Water . . . . .	0.010
Sulphuric acid . . . . .	0.23
Olive oil . . . . .	0.99
Castor oil . . . . .	10.20
Glycerin . . . . .	10.69
Pitch . . . . .	$1503 \times 10^9$
3% egg albumin . . . . .	0.012
Blood . . . . .	0.031—0.055

HEILBRONN's measurements showed that the viscosity of protoplasm might be very much less than the viscosity of glycerin or olive oil, that it might even be less than that of sulphuric acid. At the time of HEILBRONN's experiments this was rather an unusual idea, although EWART '03 had concluded from observations on the effect of gravity on rate of streaming that the viscosity of the protoplasm must be low. EWART believed that the viscosity of streaming protoplasm must lie between the limits 0.04 and 0.20, that is to say he thought it could not be less than four or more than twenty times as viscous as water. In the starch sheath cells of *Vicia faba*, the viscosity of the protoplasm is certainly less than eight times that of water, and it is probably not more than four times that of water. It should be pointed out

that HEILBRONN's values were for the entire protoplasm rather than for the intergranular material.

HEILBRONN's first measurements of protoplasmic viscosity were made in 1914. In 1920, SEIFRIZ published some estimates of the viscosity of various types of protoplasm, based on his impressions with the microdissection needle. SEIFRIZ's values are given in terms of the scale already mentioned in Chapter IV (see p. 42). The obvious discrepancies of this scale have already been discussed, and no great amount of significance can be attached to these values. SEIFRIZ believes the endosarc of a flowing ameba to have a "viscosity value" of 4, that of a quiescent ameba a "viscosity value" of 5—6. He regards active myxomycete protoplasm as "liquid", that is to say with a "viscosity value" of 3, whereas just before fruiting, he believes it to be as viscous as bread dough or vaseline. This does not agree at all with the later measurements of HEILBRONN on various species of myxomycetes, for HEILBRONN found the protoplasm just before fruiting to have a viscosity only 9 to 18.5 times that of water, depending on the species (see below).

In estimating the viscosity of marine ova, SEIFRIZ is impressed with an earlier observation of CHAMBERS '17, whom he quotes as follows: "The internal cytoplasm of a marine egg is a viscous fluid. The viscosity is high enough to prevent any Brownian movement of the inclosed granules". SEIFRIZ then cites an old observation of EXNER (made in 1867) to the effect that the Brownian movement of particles is stopped when the viscosity of the dispersion medium reaches a value as great as that of ordinary laboratory glycerin. SEIFRIZ concludes that "protoplasm in which no Brownian movement is present must possess a consistency of about this value". Accordingly he ascribes to Echinoderm ova a "viscosity value" between 6 and 7. As a matter of fact this train of argument is not very conclusive. Many authors have described Brownian movement in marine eggs. A very good description is given by LILLIE '06. CHAMBERS himself in later work ('24) mentions Brownian movement in the sea-urchin egg. As a matter of fact, in the egg of *Arbacia* the movement is not very vigorous, and for a very obvious reason. In a recent paper (HEILBRUNN '26c) it was shown that the average distance between adjacent granules of the *Arbacia* egg was approximately equal to the radius of the granules, that is to say about  $0.16 \mu$ .

On the average therefore a granule in Brownian movement in this egg can not travel farther than  $0.16 \mu$ , and this distance is so small that it is almost at the limit of microscopic visibility. Under the usual conditions, therefore, Brownian movement in the *Arbacia* egg is not very easy to see. But one has only to centrifuge the eggs, and then the Brownian movement of the granules at the edge of the granule-free area is very easy to observe and quite rapid. Indeed it has been possible to estimate the speed of this movement, as will be shown later. In view of the fact that Brownian movement does occur in sea-urchin eggs and in other marine eggs, there is no justification for SEIFRIZ's opinion that the viscosity of the protoplasm of these eggs must equal that of glycerin.

HEILBRONN '22 used the magnetic method in order to measure the absolute viscosity of slime mold protoplasm. His results are very interesting and will be considered in detail. The following table gives a summary of HEILBRONN's measurements.

Absolute viscosity of slime mold protoplasm,  
according to HEILBRONN '22

<i>Reticularia lycoperdon</i> , aethalia . . . . .	0.165—0.185
<i>Lycogala epidendron</i> , aethalia . . . . .	0.15
<i>Badhamia utricularis</i> , plasmodia before fruiting . . . . .	0.095—0.105
<i>Physarium cinereum</i> , plasmodia before fruiting . . . . .	0.090—0.115
<i>Trichia jallax</i> , plasmodia before fruiting . . . . .	0.110—0.160
<i>Dididymium serpula</i> , race I, plasmodia before fruiting . . . . .	0.090—0.095
<i>Dididymium serpula</i> , race II, plasmodia before fruiting . . . . .	0.115—0.120

The above values were obtained with slime molds on glass, surrounded by a ring of moist cardboard. If the slime molds were placed on moist filter paper and were also surrounded by a ring of moist cardboard, the viscosity decreased until it was about two-thirds of that shown in the table.

In making viscosity tests, HEILBRONN determined the number of amperes which had to be passed through an electromagnet in order to twist a small iron rod through the protoplasm. The rod was 220 microns long, and 50 microns in diameter. The number of amperes necessary to twist the rod in protoplasm was divided by the number of amperes necessary to twist it in a drop of water, and this was taken as the absolute viscosity.

HEILBRONN was very careful to test the accuracy of his method for measurements of absolute viscosity. He found that

the viscosity in small drops was always greater than that in large drops. In *Physaria* and *Badhamia*, the amount of protoplasm is small, and for this reason the viscosity values obtained for these forms is relatively high. As HEILBRONN points out, the ease with which the iron rod is twisted is not only a function of the viscosity of the fluid which surrounds it, but also a function of the surface or adhesive forces which tend to keep the rod in contact with the glass or other substratum on which it rests. The higher the viscosity the smaller the part played by these surface or adhesive forces. Hence when HEILBRONN studied the twisting of iron rods in viscous liquids of known viscosity, the values obtained for these liquids by the magnetic method were found to be too low. On the other hand, for liquids less viscous than water, too high values were obtained. Thus for a concentrated solution of glycerin, HEILBRONN obtained a value 25 % too low, and for ether a value 300 % too high.

From these considerations, in view of the fact that protoplasm is more viscous than water, we would tend to conclude that HEILBRONN's values for slime mold protoplasm were somewhat too low. But there is really another factor to be considered. An iron rod in protoplasm presumably rests on a differentiated film of protoplasm which lies between the main mass of the living substance and the glass of the slide. Protoplasm in general and slime molds in particular, form films at their boundaries (see chap. 13). These films are very likely to have more adhesive properties than glass. If this were the case, an iron rod resting on such a film would tend to have its movement seriously retarded, and too high values for viscosity would be obtained. Perhaps these two sources of error neutralize each other. But they may very possibly be of a different order of magnitude. With his electrical method, HEILBRONN found that the viscosity value obtained for a concentrated solution of glycerin was 25 % too low. Perhaps in the case of protoplasm there is a similar error of about 25 %, although this seems more or less hypothetical. It seems quite possible that the presence of a protoplasmic film beneath the iron rod might cause an error of 100 % or more.

Obviously we are more or less in the dark. HEILBRONN's values with the magnetic method are not very accurate. However, they represent results of a greater degree of precision (or a lesser degree of uncertainty), than any previously obtained. From

HEILBRONN's observations, it seems safe to conclude that the viscosity of the protoplasm of slime molds before fruiting is not over 15 to 25 times that of water, and it may be and probably is much less than that. These values are only for the endoplasm. For the ectoplasm, HEILBRONN found great variation. In some instances the viscosity of the ectoplasm approached infinity. Such variable values are to be expected if the ectoplasm is a gel, for the viscosity of a gel varies with the amount of the shearing force exerted upon it. With relatively low shearing force, the viscosity of a gel is infinity. It has already been pointed out that cells are typically surrounded by membranes, and these membranes are certainly gels. Perhaps in slime molds the ectoplasm corresponds to the membranes of ordinary cells.

In HEILBRONN's experiments with the magnetic method, he determined viscosity either by measuring the magnetic force necessary to turn an iron rod, or in some preliminary tests by measuring the magnetic force necessary to cause a barely perceptible movement of a small iron sphere. In 1924, SEIFRIZ made one or two tests of absolute protoplasmic viscosity with the magnetic method. With the aid of a micropipette, he introduced nickel balls into the eggs of the sand dollar *Echinarachnius* and then observed the speed with which they were drawn toward an electromagnet. Apparently he made but few observations, and he states only that the speed of movement of a nickel ball in an *Echinarachnius* egg is of about the same order of magnitude as the speed of movement of the nickel ball in glycerin. SEIFRIZ does not give a very complete description of his experiment, and it is hard to see how he could be certain that the nickel ball when in the glycerin was at exactly the same distance from the magnet as it was when in the egg. There are also a number of other objections that could be made to SEIFRIZ's experiment. But it is hardly worth while to consider all of them, for there is one source of error which in itself would make it impossible to draw any conclusions from SEIFRIZ's observations. In recent years it has become clearly recognized that the entrance of a needle or other object into a cell is frequently accompanied by injury. Thus PÉTERFI '27 describes various injury phenomena which follow the introduction of a fine needle into blood cells. CHAMBERS and POLLACK '27 describe injuries and so-called cytolytic changes as the result of the presence of a micropipette



in starfish eggs. These workers used needles or pipettes only a micron or two in diameter. The nickel ball used by SEIFRIZ measured 16 microns in diameter, and the pipette which introduced it into the egg must have been appreciably larger than this. Certainly it is fair to assume that the introduction of such a large foreign object would cause marked injury in any cell, and such injury might alter the viscosity in any conceivable way. By coagulating the protoplasm it might very well raise the viscosity to infinity. It would have been a simple matter for SEIFRIZ to test the extent of the injury provoked by his micropipette. It would only have been necessary for him to fertilize the eggs he had operated on, and to observe then whether or not they developed normally. There is no record of such tests in his paper.

The centrifuge method has recently been used to determine the absolute viscosity of the protoplasm of marine eggs. A description of this method has already been given, but so far little has been said regarding the physical principles on which it is based. We have seen that the movement of a granule through a fluid obeys STOKES' law. When the force acting on the granule is centrifugal force rather than gravity, the law takes the following form:

$$V = \frac{2cg(\sigma - \rho)a^2}{9\eta},$$

in which as before  $V$  is the speed of movement of the granule,  $g$  the gravity constant,  $\sigma$  the specific gravity of the granule,  $\rho$  the specific gravity of the fluid through which the granule travels,  $a$  the radius of the granule, and  $\eta$  the viscosity. In addition,  $c$  is the centrifugal force in terms of gravity.

In the above formula,  $g$  is of course known,  $a$ , the radius of the granule can be determined by direct measurement, the centrifugal force,  $c$ , can be calculated if the number of turns per second and the radius of turn of the centrifuge are known. As shown in the last chapter,  $V$  can be determined from a series of centrifuge tests. There remain then only  $\sigma$  and  $\rho$ . If it were possible to know  $\sigma$ , the specific gravity of the granule, and  $\rho$ , the specific gravity of the intergranular fluid, we would be able to use STOKES' law for the determination of the absolute viscosity of protoplasm by the centrifuge method. As a matter of fact, it is possible to determine the specific gravity of the granules of cells, and from this value it is then possible to calculate

the specific gravity of the intergranular fluid. We are thus in a position to use the centrifuge method for the measurement of absolute viscosity.

It must be remembered that the derivation of STOKES' law involves a number of assumptions. Eight of these assumptions have already been listed and more or less adequately discussed in relation to HEILBRONN's viscosity determinations on the protoplasm of starch sheath cells (see pages 56—59). In general the principles involved in the centrifuge method are much the same as those involved in the gravity method. The previous discussion of the applicability of STOKES' law is therefore in the main sufficient, and only a few additional remarks are necessary at this point. Assumption 2, that the fluid through which the granule travels is infinite in extent, has more importance for starch sheath cells in which the starch grains move, than for cells in which one follows the movement of granules. In sea-urchin eggs (*Arbacia*), the diameter of the smaller granules is only a two-hundredth of the diameter of the cell. LADENBURG's correction may therefore be neglected in this case, for it only becomes appreciable when the moving particle is much larger in comparison with the size of the containing vessel. The fifth assumption, that the velocity of the moving sphere is small, might be thought not to hold for centrifuge experiments. However, the highest speed that has ever been noted for the movement of granules through a cell under the influence of centrifugal force is less than  $2 \times 10^{-3}$  cm. per second. Such a speed is certainly small enough so that its higher powers may be neglected, and this is the condition required for the validity of STOKES' law. Moreover, in the cell in question (the *Cumingia* egg), centrifuge tests with a relatively small amount of centrifugal force give the same viscosity values as when the granules move at top speed with higher centrifugal force.

Finally attention should be directed again to assumption 7, that there is only one sphere or granule moving through the fluid. In living cells there are vast numbers of granules, and all of them move when the cells are centrifuged. Fortunately we can apply the correction factor of CUNNINGHAM. In order to use this correction, we need merely know the radius of the granules, and the distance between them. However, CUNNINGHAM's factor considers only the case in which granules of the same size are moving in the same direction. Many cells contain

granules of differing size, or they contain granules which move in opposite directions on the application of centrifugal force. Granules of different sizes would move at different rates of speed, they would thus tend to collide. Collisions would also occur between granules moving in opposite directions. The effect of all these collisions would be to decrease the speed of the moving granules, and this would make the values for the viscosity obtained by the centrifuge method higher than they really should be. Because of the varying conditions found in different cells, it would probably be best to consider the effect of collisions for each type of protoplasm separately. In those cases in which practically all of the granules are of one size and move in the same direction, the collision factor may be neglected.

We are now in a position to consider the actual measurements. In the *Arbacia* egg the viscosity was determined both from the speed of the small heavy granules and from the speed of the larger pigment granules which are also heavier than the rest of the protoplasm. Let us consider first the case of the smaller granules. Inasmuch as these granules are very much more abundant than the other granules in the egg, we may neglect the influence of collisions, especially in view of the fact that collisions with the larger granules would tend to increase the speed of movement, whereas collisions with the fat or lipid granules would tend to decrease it.

In order to apply STOKES' law, we must find values for  $V$ ,  $a$ ,  $\sigma$ ,  $\rho$  and  $g$ . The determination of the velocity,  $V$ , is a relatively simple matter. In a series of tests, one finds the number of seconds just sufficient to move the small heavy granules into one half of the egg. When nearly all of the granules are in one half of the egg, we can assume that the granules originally at the pole farthest distant from this half are now at the equator. The distance traveled is thus half the diameter of the egg, and when this is divided by the time in seconds required for the movement, we obtain  $V$ . As a matter of fact, the distance travelled is slightly greater than the radius of the egg, for during centrifugal treatment the egg becomes somewhat elongated. If this elongation is taken into account, the speed of the granules is found to be 0.00011 cm. per second, when the centrifugal force is 4968 times gravity.

The radius of the granules,  $a$ , is obtained by direct measurement with a movable scale ocular. For very small granules

such measurement is certainly inaccurate, and the inaccuracy is especially important in view of the fact that the value for  $a$  is squared in the formula. The radius of the small heavy granules in the *Arbacia* egg was found to be  $0.16\mu$ , or  $0.000016$  cm.

In order to determine the specific gravity of the granules, the eggs were broken up by shaking them with splintered glass, and the resultant mass was then centrifuged in various concentrations of sugar solution. In concentrated solutions of sugar the granules move to the inner part of the centrifuge tube, in dilute solutions they go to the bottom, that is to say to the outer part of the tube. Those solutions of sugar in which the granules move neither the one way or the other, have the same specific gravity as the granules. Inasmuch as the specific gravity of various concentrations of sugar solution is known, it is possible to determine the specific gravity of the granules. But this determination rests on the assumption that the granules do not change their specific gravity after emerging from the egg. In all the end determinations, the specific gravity of the granules was tested as rapidly as possible after their removal from the egg. However, if the granules were allowed to remain out of the egg for a time, the specific gravity did not change from the value obtained immediately after removal. This is evidence that the specific gravity of the granules outside of the egg is not markedly different from their specific gravity within the egg. But it is far from conclusive evidence, for it is quite possible that as the granules emerge there is a very rapid change in their specific gravity. Here then is a source of error, and apparently we have no means of knowing how great an error may be involved. But fortunately there is a method of calculating the maximum value which the specific gravity of the granules may have, so that we can at least limit the extent of the possible error in one direction. As we shall see later, there is no need of limiting it in the other direction. For the present, we will be content with the value obtained in the manner already outlined.

It is possible to determine the specific gravity of entire eggs in the same way that the specific gravity of the granules was determined, that is to say by centrifuging them in various strengths of sugar solution. Knowing the specific gravity both of the eggs and of the granules, it is possible to calculate  $\rho$ , the specific gravity of the intergranular fluid, provided that one knows the

relative volume of the granules as compared to the volume of the entire egg. The relative volume of the granules can be computed from the fact that after the most vigorous centrifugal treatment the granules occupy a spherical segment, the altitude of which is about a third of the diameter of the egg. From simple geometrical considerations, it can then be shown that the granules occupy  $\frac{3}{16}$ ths of the total egg volume. Knowing this fraction, it is easy to derive a simple equation for  $\varrho$  in terms of  $\sigma$ , the specific gravity of the granules, and  $s$ , the specific gravity of the entire eggs:

$$\varrho = \frac{16s - 3\sigma}{13}.$$

By calculating  $\varrho$ , it is possible to arrive at a value for  $\sigma - \varrho$ . For further details, see HEILBRUNN '26c.

It only remains to find  $q$ , the CUNNINGHAM correction for STOKES' law. This correction, as shown previously, involves both the radius of the granules and the distance between the centers of adjacent granules. The radius is known, the distance between the centers of adjacent granules can either be measured, or it can be computed from the proportion of the total volume of the egg that the granules occupy. Such computation is certainly more accurate than direct observation could be. The distance between adjacent granules is calculated in the following fashion. We assume that the granules are surrounded by spheres, so that the entire volume of the egg is filled. Then if the radius of the granules is  $a$ , and the radius of the spheres is  $b$ , and the total number of granules or spheres  $n$ , the volume of the egg is  $n \times \frac{4}{3}\pi a^3 \times \frac{16}{3}$ , since we have already shown that the volume of the granules is  $\frac{3}{16}$ ths of that of the entire egg. The volume of the egg is also equal to  $n \times \frac{4}{3}\pi b^3 \times \frac{4}{3}$ . (The volume of the spheres must be multiplied by  $\frac{4}{3}$ , since spheres when closely packed occupy only 74.05 % of a given volume.) Equating the two equations for the volume of the egg, and solving for  $b$ , we find  $b = a\sqrt[3]{4}$ . Knowing  $a$  and  $b$ , we can calculate  $q$  from the equation given on p. 58.

We now have all the terms necessary. Substituting them into the modified form of STOKES' law, the viscosity is found to be 0.0183. This is 1.8 times the viscosity of water.

The viscosity of the *Arbacia* egg protoplasm may also be

calculated from the speed of movement of the large pigment granules. These granules have a radius of 0.46 microns or 0.000046 cms. With a centrifugal force 4968 times gravity, their speed is about 0.00024 cms. per second. Ordinarily the pigment granules break down when they emerge from the egg, and the measurement of their specific gravity would involve difficulties. The pigment granules do not break down in the absence of calcium (see chap. 13), and perhaps by smashing the eggs in calcium-free sea-water, a value for the specific gravity of the pigment granules might be obtained. This was not done, and in the absence of a measured value, we may assume that the pigment granules have the same specific gravity as the colorless granules.  $\rho$  and  $q$  are thus the same for the pigment granules as for the colorless granules. Substituting into the modified form of STOKES' law, we now obtain a value of 0.069 for the viscosity. This value is too high, because the effect of collisions has been neglected. The large pigment granules as they move through the egg are continually colliding with the smaller granules which move at a much slower rate. The retarding effect of these collisions can be calculated, for details see HEILBRUNN '26c. When correction is made for the effect of collisions, the value for the viscosity, as determined from the speed of the pigment granules, is 0.025, or 2.5 times that of water. This value agrees fairly well with the one previously found from the speed of movement of the small granules. We therefore conclude that the viscosity of the intergranular material of the *Arbacia* egg is approximately two times that of water.

There are a number of possible errors involved in the measurement of the viscosity of *Arbacia* protoplasm. The most important of these are:

1. The error due to the inexact determination of the size of the small granules.
2. The possible error involved in a change of specific gravity of the granules which may occur as they emerge from the egg.
3. The error involved in the calculation of  $q$ .
4. The possible error due to an influence of the moving granules on the viscosity.

The last of these sources of error really does not exist. It is true that in many gels, pressure or mechanical force can produce a lowering of the viscosity. But in the *Arbacia* egg no such

effect is present, for when measurements of viscosity are made with widely different amounts of centrifugal force, the same values are obtained. If the movement of the granules through the protoplasm produced a lowering of the viscosity, then the greater the centrifugal force and the greater the speed of the granules, the lower the viscosity would be.

The first source of error, that due to the inexactitude with which the size of the granules is measured, may be considerable. The pigment granules of the *Arbacia* egg are large enough so that their diameter may be determined with some degree of accuracy. But the colorless granules are too small for accurate measurement. One way of estimating the size of the colorless granules is to compare them with the pigment granules. Their diameter appears to be about one-third as great, and this estimate of their size agrees very well with the value obtained by direct measurement with a movable scale ocular. If instead of being a third, the diameter of the small granules were as much as a half of the diameter of the pigment granules, then the viscosity value given above would be only one-half as great as it should be. However it does not seem possible that the diameter of the small granules could possibly be as great as one-half that of the pigment granules, so that this is the maximum error possible.

The second possible error appears in the determination of the specific gravity of the granules. Perhaps they undergo a change in specific gravity as they emerge from the egg. If the specific gravity of the granules decreased on emergence, then the value obtained for the viscosity would be too low. It is hardly necessary to consider the opposite possibility, that the granules undergo an increase in specific gravity, for no one could assume that the value obtained for the viscosity is much too high. It is too close to the viscosity of water. In computing the viscosity, the value taken for  $\sigma - \rho$ , the difference in specific gravity between the granule and the intergranular fluid, was taken as 0.716. Now it can be shown by a simple calculation that this can not be far from the true value. We know the specific gravity of the entire egg, it is approximately 1.05. We know further that the colorless heavy granules occupy about  $\frac{3}{16}$ ths of the total volume of the egg. If then we knew the specific gravity of the intergranular fluid, we could calculate the specific gravity of the colorless heavy granules. The specific gravity of the intergranular fluid

can not vary within very wide limits. It must be greater than the specific gravity of sea-water, for it contains at least as much salt as sea-water and probably more (compare p. 25), and in addition it contains dissolved proteins. Now if we assume that the specific gravity of the intergranular fluid is *equal* to that of sea-water, that is to say 1.03, then the specific gravity of the granules is found by simple algebra to be 1.14, and  $\sigma - \rho$  is 1.1. This is certainly a maximum value, and yet it is not very much greater than the value 0.716 used in computing the viscosity. If we were to assume that the specific gravity of the intergranular fluid were only slightly greater than that of sea-water, then decidedly lower values of  $\sigma - \rho$  would be found by computation. Thus if the specific gravity of the intergranular fluid were taken as 1.04, then  $\sigma - \rho$  would be only 0.5. In these calculations we have neglected the pigment granules and the fatty granules. This involves no serious error, for their total volume is very small compared to the volume of the colorless heavy granules. Our calculations show beyond any question that the value used for  $\sigma - \rho$  is not far from the correct one. The second of the possible errors is therefore not great.

Finally there is the error involved in the calculation of  $q$ . CUNNINGHAM's mathematical treatment involves a number of assumptions, and his formula for  $q$  is not exact. How great an error may be involved, it is at present impossible to say.

The question of the possible errors involved in the determination of the absolute viscosity of the protoplasm of the sea-urchin egg has been taken up in some detail. This has been done in part because it has seemed impossible to some commentators that any protoplasm could be so fluid. One reviewer has indeed pointed out the contradiction between the value obtained by the centrifuge and that arrived at by SEIFRIZ. It will be remembered that SEIFRIZ was of the opinion that the viscosity of the sea-urchin egg protoplasm was like that of glycerin and he thinks of it as being about 800 times that of water. The value obtained by the centrifuge method is of a totally different order of magnitude, even if we allow for every possible source of error and go so far as to neglect the CUNNINGHAM correction completely. Because of the sharp contrast between the values of HELBRUNN and SEIFRIZ, it is of interest to consider the determination of the viscosity of Arbacia protoplasm by another method.



When one centrifuges *Arbacia* eggs, the heavy granules pass into one hemisphere of the egg. They do not remain in this position indefinitely, and after a time they again become scattered through the entire egg. The return of the granules is due to Brownian movement. According to EINSTEIN, the distance travelled by a granule in Brownian movement in the time  $t$  is given by the following formula:

$$D_x^2 = 12.6 \times 10^{-18} \frac{Tt}{\eta a} *$$

In this formula  $D_x$  is the distance travelled by the granule in any one plane,  $T$  is the absolute temperature,  $a$  the radius of the granule, and  $\eta$  the viscosity. In the case we are considering, we already know  $a$  and  $T$ .  $D_x$  may be taken as the radius of the egg, that is to say approximately 38 microns. If now we can determine  $t$ , the time required for a granule to travel across half of the egg, we could calculate the viscosity. No very exact determination of  $t$  is possible. But it is a simple matter to find the general order of magnitude of  $t$ , and from this the general order of magnitude of the viscosity. In the following table,  $t$  has been calculated for various assumed values of  $\eta$ . For the sake of simplicity, we have used  $\eta_w$ , the viscosity in terms of water ( $\eta_w = 100 \eta$ ).

$t$	$\eta_w$
150 hours	859
100 "	573
50 "	286
25 "	143
10 "	57
5 "	28.6
2 "	11.46
1 "	5.73
$\frac{1}{2}$ "	2.87

According to the table, if the viscosity of *Arbacia* egg protoplasm were eight or nine hundred times water as SEIFRIZ believes it to be, it would require a hundred and fifty hours for the granules to return through the egg after being centrifuged into one hemi-

\* See BURTON, Physical properties of colloidal solutions. 2nd edition, London and New York 1921.

sphere. Ordinarily the *Arbacia* egg only remains alive for twenty-four hours. And surely within two hours after centrifugal treatment the granules have become completely scattered throughout the egg. If one tries to estimate the time required for the first granules to reach the pole of the egg farthest from the hemisphere to which they have been centrifuged, one finds that no exact measurement is possible. The time seems to be more than half an hour and less than an hour. This would indicate a viscosity between 2.87 and 5.73 times that of water, or roughly about four times that of water. It must be remembered that the EINSTEIN formula is not exact, and that it would probably give too high values for the viscosity when the granules are as numerous as they are in sea-urchin eggs. With a high concentration of granules, there is surely a retardation of speed of Brownian movement, although this might not be noticeable if the granules are moving toward a granule-free area.

The study of Brownian movement in *Arbacia* eggs has shown that SEIFRIZ's value for the viscosity of the protoplasm is certainly wrong. This was to be expected, for SEIFRIZ's opinion was primarily based on the erroneous observation of CHAMBERS to the effect that there is no Brownian movement in marine eggs (see previous discussion).

The determination of the protoplasmic viscosity of the *Arbacia* egg, both by the centrifuge method and by the study of Brownian movement, depends on the measurement of the small granules. These can not be very accurately measured, as has already been pointed out. Fortunately any error in the measurement of the granules would have opposite effects in the two determinations. Thus, if the value that we have used for the radius of the granules is too low, this would result in too low values for the viscosity as determined by the centrifuge method and in too high values for the viscosity as determined by the Brownian movement method. In view of this fact, it seems wise to average the results obtained by the two methods. In this way, we find the most probable value for the absolute viscosity of the *Arbacia* protoplasm to be 0.03, or three times the viscosity of water.

It seems certain that the viscosity of the *Arbacia* egg protoplasm, or rather of the non-granular part of the protoplasm is only several times as great as that of water. To biologists used

to thinking of protoplasm as a very viscous fluid, this may seem surprising, but from a physical standpoint it is readily understandable. The non-granular part of protoplasm is essentially a protein solution (at any rate the constituents which would have the greatest effect in increasing viscosity are proteins). Now most protein solutions either have a viscosity two or three times that of water, or they are gels with a viscosity approaching infinity. The viscosity measurements indicate certainly that the protoplasm of the *Arbacia* egg is not a gel, and in the next chapter additional evidence will be presented which will bear out this conclusion. It is therefore not at all surprising that the non-granular protoplasm of the *Arbacia* egg should have a viscosity of the same order of magnitude as a protein sol.

The determination of the absolute viscosity of *Arbacia* egg protoplasm by the centrifuge method was to some extent uncertain because of the possible error involved in  $q$ , the correction factor of CUNNINGHAM. In the egg of the clam *Cumingia*, this factor is of less importance. This is due to the fact that in the *Cumingia* egg, there are almost as many light granules as heavy granules (see Fig. 1, p. 45). Hence when the egg is centrifuged and the heavy granules move in one direction, it is comparatively easy for a return flow to accompany the lighter granules in the opposite direction. In the determination of the protoplasmic viscosity of *Cumingia* egg protoplasm, the CUNNINGHAM factor was neglected. The results obtained were thus certainly too high. They are also too high for another reason. The *Cumingia* egg contains two sets of granules which move in opposite directions when the egg is centrifuged. These granules would certainly tend to collide, and the collisions when they occurred would slow the speed of the granules and thus tend to increase the viscosity values. The effect of such collisions was also neglected.

The specific gravity of the heavy granules of the *Cumingia* egg was determined by the same method employed for the similar granules of the *Arbacia* egg. The value obtained was 1.18677. The calculation of the specific gravity of the intergranular fluid is relatively simple. The *Cumingia* egg contains approximately equal numbers of light and heavy granules, and these are not only of the same approximate size, but they differ in specific gravity from the intergranular fluid by approximately the same amount. For when the egg is centrifuged, the light granules

travel about as fast in one direction as the heavy granules travel in the other. From these considerations, it is apparent that the specific gravity of the entire egg must be approximately equal to that of the intergranular fluid. The specific gravity of the entire egg was found to be 1.04853.  $\sigma - \rho$  is therefore 1.18677 — 1.04853, or 0.13824. Here again there is a possible error, as in the case of the *Arbacia* egg, for the granules may change their specific gravity upon emergence from the egg. Fortunately, for the *Cumingia* egg it is possible to show that errors in the determination of the specific gravity of granules can not be of such a nature as to make the values found for the viscosity much lower than they should be. It is certain that the specific gravity of the intergranular fluid is 1.04853. Now the light granules can not have a specific gravity much lower than 0.9, for there are no substances which could possibly be present as fluid or solid material in protoplasm which could have a specific gravity more than a few hundredths lower than this. If we assume the specific gravity of the light granules to be 0.9, then  $\sigma - \rho$  for the light granules is 1.04853 — 0.9 or 0.14853. This value is only slightly greater than that already found for the heavy granules. As stated previously  $\sigma - \rho$  must be the same for light granules and heavy granules. Hence the value found for the heavy granules can not possibly be more than a few percent too low.

The granules of the *Cumingia* egg move very rapidly under the influence of centrifugal force (for details see HEILBRUNN '26c). From the speed of this movement one can calculate the viscosity of the protoplasm. It is found to be 0.043, or 4.3 times that of water. This is really a maximum value. As stated previously, no allowance was made for CUNNINGHAM's correction or for the effect of collisions between granules. Both of these factors would tend to slow the speed of the granules, and both would make the values determined for the viscosity higher than they should be. All that we can say with certainty is that the viscosity of the intergranular *Cumingia* protoplasm lies between 1 and 4.3 times that of water.

The determination of the viscosity of the *Cumingia* protoplasm is especially important in that it seems that all possible errors which would tend to markedly increase the value have been excluded. CUNNINGHAM's factor has been completely neglected, the specific gravity of the granules is certainly not appre-

ciably greater than we have found it to be, and even if the size of the granules is somewhat larger than our measurements indicate, this can not be a very important source of error. Apparently beyond any question the viscosity of the protoplasm of the *Cumingia* egg is of the same order of magnitude as that of a protein sol.

Both in the case of the *Cumingia* egg and the *Arbacia* egg, our determinations of protoplasmic viscosity concern only the material between the granules, that is to say the dispersion medium of the protoplasmic suspension. It is of interest now to consider the viscosity of the entire suspension, granules plus dispersion medium. The viscosity of a suspension may be calculated according to the formula derived by EINSTEIN\*, and later by HATSCHEK\*\*. This formula may be written as follows:

$$\eta_c = \eta_o (1 + kf),$$

in which  $\eta_c$  is the viscosity of the suspension;  $\eta_o$ , the viscosity of the dispersion medium;  $f$ , the ratio of the volume of suspended particles to the total volume of the suspension; and  $k$  is a constant. EINSTEIN gives the value of  $k$  as 2.5, HATSCHEK considers it to be 4.5. In the protoplasm both of the *Arbacia* and of the *Cumingia* eggs,  $f$  is about  $1/5$ . The formula therefore shows that the viscosity of the entire protoplasmic suspension is one and one-half to two times the viscosity of the intergranular fluid, depending on whether one uses EINSTEIN's value for  $k$  or HATSCHEK's.

It is now generally recognized that the formula of EINSTEIN and HATSCHEK does not give very accurate results, especially for more concentrated suspensions. Various other formulae have been suggested. Thus, for example, BINGHAM\*\*\* has proposed the following empirical formula:

$$\eta_c = \frac{\eta_o}{\left(1 - \frac{f}{d}\right)}.$$

$\eta_c$  and  $\eta_o$  are as before,  $f$  is the volume concentration of the solid, and  $d$  is the particular value of  $f$  at which the concentration of the granules is so high that the viscosity becomes infinite.

\* EINSTEIN 1906, Ann. d. Physik, ser. 4, vol. 19, p. 289, 1911, vol. 34, p. 591.

\*\* HATSCHEK 1911, Kolloid-Zeitschr., vol. 7, p. 301.

\*\*\* BINGHAM, Fluidity and plasticity, New York 1922.

Perhaps BINGHAM's formula is better than that of EINSTEIN and HATSCHEK. As the concentration of suspended matter becomes higher and higher, the viscosity of the suspension certainly must approach infinity, and this could not be predicted from the EINSTEIN-HATSCHEK formula. But in order to use BINGHAM's formula, one must know  $d$ , the concentration of suspended material at which the viscosity becomes infinite. For inanimate suspensions,  $d$  can readily be determined by varying the concentration of suspended matter until there is no longer any flow possible. At first sight it would not seem feasible to apply the BINGHAM formula to protoplasm, for one can not add or take away granules from the cell. And yet it is really a very simple matter to change the granular concentration of the protoplasmic suspension. Osmotically one can extract water from the cell, and of course this has the effect of increasing the concentration of granules. When *Arbacia* eggs are placed in a hypertonic solution which reduces the volume to three-fourths of what it is normally, the viscosity of the protoplasm is increased about six-fold. When the volume is decreased to two-thirds of the normal, the viscosity is greatly increased and approaches infinity. Similar results were also obtained for *Cumingia* eggs. For both types of material therefore, the value for  $f/d$  in BINGHAM's formula is approximately  $\frac{2}{3}$ . From this figure, we can calculate the viscosity of the protoplasmic suspension in both *Arbacia* and *Cumingia* eggs as three times the viscosity of the intergranular fluid.

The application of BINGHAM's formula is not without error. The increase in protoplasmic viscosity in hypertonic solutions is apparently not due entirely to the greater proximity of the granules. For when the eggs are returned to sea-water, the viscosity does not completely return to its original low value. There is thus an error due to the specific action of hypertonic solutions in increasing the viscosity. The effect of this error would be to make the viscosity as calculated from BINGHAM's formula somewhat too high. If we average the results from the EINSTEIN-HATSCHEK formula and from the BINGHAM formula, we can conclude that the viscosity of the protoplasmic suspensions in *Arbacia* and *Cumingia* eggs is  $2\frac{1}{2}$  times that of the intergranular fluid.

In the *Arbacia* egg, there is also a direct method of estimating the viscosity of the entire protoplasm. In this egg, it will be

remembered that the heavy granules are of two sorts, small colorless ones, and pigment granules with a diameter approximately three times as great. The pigment granules are very few in number as compared with the colorless granules. When the eggs are centrifuged, the pigment granules, because of their larger size, tend to move much more rapidly than the colorless granules. But the more rapid movement of the pigment granules is continually retarded by collisions with the colorless granules in their path. We have already considered the effect of these collisions in calculating the viscosity of the intergranular fluid from the speed of the pigment granules. Viewed from another angle, the speed of movement of the pigment granules through the rest of the protoplasm is a measure of the viscosity of the rest of the protoplasm, and inasmuch as the pigment granules are few, the viscosity of the rest of the protoplasm can not differ very appreciably from the viscosity of the entire protoplasm. From this point of view, if we neglect the effect of collisions, the viscosity as calculated from the speed of movement of the pigment granules should give a rough measure of the viscosity of the entire protoplasm. It has already been pointed out (see p. 79), that the viscosity calculated in this manner is 6.9 times that of water. The viscosity of the intergranular protoplasm of the *Arbacia* egg was found to be three times that of water. From the speed of the pigment granules, one would conclude that the viscosity of the entire protoplasm was two and a half times that of the intergranular material. The value obtained by direct determination thus agrees perfectly with the value obtained by calculation from the EINSTEIN-HATSCHEK and BINGHAM formulae.

The entire protoplasm of the *Arbacia* egg is thus about seven times as viscous as water, and the entire protoplasm of the *Cumingia* egg is something less than eleven times as viscous as water. It is certain therefore that the viscosity of the protoplasm of these marine eggs is of the same order of magnitude as that of the plant cells studied by HEILBRONN. The viscosity of the protoplasm of these cells is less than that of an oil, less than that of sulphuric acid. Ordinarily as the biologist examines protoplasm under the microscope, he gets the impression that it is very viscous. "It is probable that such an impression, doubtless a universal one, is due in part to an illusion. When we examine

protoplasm under the microscope, we see granules much larger than they really are. They may be magnified a hundred, five hundred, a thousand times. Often as the cell is subjected to outside forces, we follow the movement of these granules from one part of the cell to another. Now the movement of a particle through a fluid under a given force is faster the larger the particle. For small particles, the speed varies as the square of the diameter. We see that the granule we are observing appears to move with some difficulty, we think of it as a large granule, failing to realize that it is much magnified, and subconsciously we associate the difficulty of motion with a high viscosity. We do not take account of the fact that the granule is really very tiny. Of course, it is true that the speed of the granule is magnified as well as its size, but it must be remembered that the ease with which a granule moves varies as the square of the diameter. A granule which is only one-hundredth of its apparent size would move at a speed one ten-thousandth of what we might expect if it were as large as it looked, or one-hundredth of what we might expect if not only the granule size, but the speed as well were magnified by the microscope. In our minds we naturally correlate the slow movement of the seemingly large granules with a viscosity much greater than it really is." (HEILBRUNN '26c.) Again when we see protoplasm flow through narrow tubes, both the speed of flow and the diameter of the tube are magnified equally. But by POISEUILLE's law the speed of flow increases with the fourth power of the diameter of the tube. If we saw the tube as small as it really is, instead of magnified hundreds of times, we should doubtless subconsciously associate any flow through such a narrow tube with a very low viscosity.

Actually we are concerned not so much with the explanation of impressions or illusions as with actual facts. And at the present time the best measurements available indicate that the protoplasm of starch sheath cells, of slime molds, and of marine eggs is only a few times greater than that of water.

It must not be supposed that all living cells have such a low protoplasmic viscosity. Certainly this is not true when the entire protoplasm is considered. Some muscle cells are composed for the most part of fibrils, definite formed elements which practically fill up the cell. The presence of these fibrils must result in a certain degree of rigidity. The contents of the muscle cell,



considered as a whole, must have a very high viscosity, although the fluid between the fibrils may or may not be very viscous. Probably many types of specialized cells contain fibrillar strands of one sort or another. Thus for example in the common protozoan paramecium, there is a neuromuscular apparatus composed of strands which extend through the ectoplasm and also ramify into the endoplasm. Also in this protozoan there are strands in the endoplasm along which the food vacuoles travel\*. The presence of these strands would have an effect on the viscosity of the protoplasm as a whole, and they might offer some resistance to the passage of granules or food vacuoles through the cell.

It is not surprising therefore that FETTER<sup>25</sup> found high viscosity values for the protoplasm of paramecium. In her experiments, she fed the paramecia powdered iron and then centrifuged them. On the basis of the speed of the food vacuoles filled with the powdered iron, she calculated the viscosity of the protoplasm of the paramecium. In making this calculation she determined the specific gravity of moist iron particles, and she assumed that the specific gravity of the fluid through which the iron vacuoles passed was approximately equal to the specific gravity of the entire paramecium. As a result of the calculation, it was found that the viscosity of the paramecium protoplasm, considered as a whole, was 87.26, or 8726 times that of water. In another experiment, FETTER fed the paramecia starch and then determined the speed of movement of the food vacuoles filled with starch grains. In this test the viscosity was found to be 8027 times that of water. It is very probable that the viscosity of the paramecium protoplasm varies with different rates of shear. It would be interesting to make determinations of absolute viscosity at different centrifugal speeds.

It has been intimated that the presence of strands in the paramecium cell would make the viscosity of the protoplasm as a whole high. This is a logical explanation of the high viscosity values obtained by FETTER, but it is of course not the only explanation, and as a matter of fact it does not seem to be the most probable one. If a paramecium cell is centrifuged even for long times and at high speeds, the small granules which are scattered throughout the protoplasm do not move at all. This may be

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\* BOZLER 1924, Zeitschr. f. verg. Physiol., vol. 2, p. 82.

due to any one of three causes. Perhaps the intergranular medium is too viscous to permit of a movement of the granules, or perhaps the granules do not differ very markedly in specific gravity from this medium, or perhaps finally the cell is so full of granules that there is no possibility of their moving into a limited portion of the cell. This last explanation seems the most probable one. Apparently in many cells the granules are so numerous that they fill up almost the entire volume. This is apparently true in the case of the egg of the starfish *Asterias*. After long centrifugal treatment only a very small region of the egg becomes free of granules. And longer centrifugal treatment does not seem to increase the extent of this granule-free region. This was probably first noted by LYON '07. Similarly in the case of the egg of the sea-urchin *Stronglyocentrotus*, it was found impossible to centrifuge the granules into a small fraction of the egg volume. It is not surprising therefore that GURWITSCH '05 found these eggs very resistant to centrifugal force. After long centrifuging, there is indeed a small region which becomes free of granules, but the volume of this region is very small. It may sometimes be seen as a little hyaline projection at one end of the egg.

In cells in which the granules are so numerous that they occupy practically all of the space, one must conclude that the viscosity of such a concentrated protoplasmic suspension is high. It would certainly be very surprising if it were not. As to the viscosity of the intergranular medium of such cells, we have no precise information. But the fact that the granules are so closely crowded and yet in living cells must retain the power of moving over each other is a strong indication that the viscosity of the intergranular medium must be low.

In this chapter it has been shown that in certain cells at least, the viscosity of the granule-free protoplasm is only several times as great as that of water. Even when the granules are considered along with the rest of the protoplasm, the viscosity of the entire suspension is often not more than eight or ten times that of water. These values are much lower than those usually assumed. Thus in a recent book (MEYER '21), the author assumes as a matter of course that the viscosity of the streaming protoplasm of plant cells is well over that of castor oil, that is to say over 1000 times that of water. Other authors assume that protoplasm is typically a gel with high viscosity.

It is hoped that in the future the viscosity of the protoplasm of many other types of cells will be measured. In many instances the determination of the absolute viscosity of the protoplasm is not a very difficult matter. Often the specific gravity of the granules of a cell can be calculated in the manner outlined for the *Arbacia* egg. The difference between the specific gravity of the granules and that of the rest of the protoplasm can then be set within fairly narrow limits, and when this is done, merely a few centrifuge tests together with a few simple size measurements are necessary to complete the tests.

The discussion so far has been concerned only with the viscosity of the cytoplasm. In view of the fact that the nucleus is such an essential part of the cell, it is very important to know what the physical properties of its interior are. Unfortunately, there is very little definite information.

In the literature of the present time, one finds many opinions as to whether the interior of the nucleus is a sol or a gel, but there is not much evidence one way or the other. LEPESCHKIN '24 believes that the interior of the nucleus is a sol. So does SCHAEDE ('26), although he had previously held ('25) that it might sometimes be a gel.

KITE '13 studied the nuclei of a number of types of cells with the microdissection method. He found all the nuclei tough, with the exception of the nucleus of the immature starfish egg. CHAMBERS '24 believes that KITE's results are due to coagulation or gelatinization following the entrance of the needle, and he cites his previous observations, and the unpublished observations of CHAMBERS and SCHMITT, to show that the nucleus has a fluid interior. According to CHAMBERS, "With improved methods, it has definitely been shown, however, that the interkinetic nucleus in every living metazoan cell so far studied is fluid and possesses no visible structure except for one or several nucleoli and a delicate investing membrane". TAYLOR '20 found by microdissection that the macronucleus and micronucleus of the protozoan *Euplotes* were both gels. He states that the micronucleus could be pulled into threads. Similar observations were made by BERTHOLD '86 (see p. 48) for the nuclei of the pollen mother cells of various monocotyledenous plants. These also could be pulled into long threads.

In view of the many objections to the microdissection me-

thod (see p. 40), it is doubtful if the results obtained by microdissection are of any value. They have unfortunately drawn attention away from definite exact observations on uninjured cells, which are of course far more valuable. These observations are of two sorts, those on Brownian movement of particles within the nucleus, and those on the movement of the nucleolus through the nucleus under the influence of gravity or of centrifugal force.

Before discussing the observations on Brownian movement, it should be clearly pointed out that CHAMBERS' statement that there are no visible structures in the interior of the living nucleus other than the nucleoli is certainly not true generally, for it is contradicted by the observations of dozens, probably hundreds of observers. The first books on the cell, for example those of SCHWANN '39, SCHLEIDEN '49, and HOFMEISTER '67, contain descriptions of granules visible within living nuclei, and they have been described by numerous observers since then.

The granules within living nuclei may show Brownian movement. Russo '10 barely mentions movements of granules within nuclei. The most complete study of the Brownian movement of granules within living nuclei has been made by GROSS '16. He observed chiefly four types of cells. The nuclei of the cells of the salivary gland of the snail *Limnea stagnalis* have many granules which are in very active Brownian movement. As is typical for Brownian movement, the amplitude of the displacements decreases as the granular diameter increases. For granules with a diameter over  $4\mu$ , Brownian movement is no longer visible. Granules  $1\mu$  in diameter suffer displacements of  $1\mu$ . GROSS states definitely that the displacement may occur in any plane in space, an important point. GROSS also describes Brownian movement of visible granules in the nuclei of clam eggs and in the nuclei of the epithelial cells of salamander larvae. GROSS describes threads or filaments in the nuclei of the Malpighian tubes of *Corethra* larvae. He does not mention any movement of these filaments and they were probably motionless. However, SHIWAGO has described motile filaments in the resting nucleus of frog leucocytes. LEPESCHKIN '24b describes Brownian movement in the nuclei of various plant cells.

From GROSS' observations, it is possible to get a general idea as to the viscosity of the intergranular fluid of the nucleus. For the nuclei of the salivary gland cells of the snail, GROSS

states that granules 1 micron in diameter suffered displacements of  $1\ \mu$ . Unfortunately GROSS does not state how great a time was required for such a displacement. If he had recorded this time, it would have been possible by the use of the formula given on page 73 to calculate the viscosity. Thus if the time were one second, the viscosity would be 0.07, if the time were 10 seconds, the viscosity would be 0.7. It is very probable that GROSS made his observations quickly, and thus it seems certain that the viscosity of the nuclear fluid of snail cells is not much greater than the viscosity of water.

Various botanists have noted that when plant cells are centrifuged, the nucleolus behaves as if it were heavier than the rest of the nuclear material and is thrown to the centrifugal end of the nucleus (see for example MOTTIER '99, ANDREWS '03, '15). Often the nucleus is so forcibly moved that it breaks through the nuclear membrane. In animal cells the nucleolus is also moved by centrifugal force, at any rate this is true in the case of immature eggs with large germinal vesicle (see LILLIE '06). In oocytes, the nucleoli move under the influence of gravity. Thus HERRICK '95 in studying sections of the ovary of the lobster, noted that the nucleoli were always orientated toward the lower side of the nucleus. In the immature egg of the sea-urchin *Echinus esculentus*, GRAY '27 has measured the speed with which the nucleolus falls under the influence of gravity. It travels at the rate of  $0.4\ \mu$  per second. GRAY does not state the size of the nucleolus, but from his figures it can be seen to be about  $16\ \mu$ . If now we make the assumption that the difference between the specific gravity of the nucleolus and the nuclear fluid is 0.1, the viscosity of the nuclear fluid as calculated from STOKES' law is 0.02 or two times the viscosity of water. This value must be very near the correct one. Obviously it can not be much too high. And it can not be much too low, for the only value about which there is any doubt is the value we have taken for the difference in specific gravity between nucleolus and nuclear fluid. The specific gravity of the nuclear fluid must be about 1.04 (that of sea-water is 1.03). It would hardly be possible for the specific gravity of the nucleolus to be very much greater than 1.14, for there are no substances in protoplasm which have a much higher specific gravity. The specific gravity of proteins typically does not exceed 1.3.

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## CHAPTER VI

# THE ELASTICITY OF PROTOPLASM

When a solid body is distorted, it tends to regain its original shape, i. e. it is elastic. Liquids and gases when compressed into a smaller volume, expand to their original volumes when the pressure is released. They thus exhibit volume elasticity, but they do not ordinarily possess elasticity of form. However thin films of liquid do regain their form following distortion, a soap bubble subjected to pressure returns to its original shape when the pressure is released. As is well known a surface tension film can behave like an elastic membrane. Moreover, even in mass, solutions which we ordinarily regard as fluid may occasionally show form elasticity. Thus a gelatin sol may have elastic properties in spite of the fact that it flows fairly easily. In the case of the elastic gelatin sol, its fluidity or viscosity is a function of the pressure or shearing force to which it is subjected. If the pressure is low enough, there is no flow at all. Only when a certain initial resistance is overcome, does the sol exhibit the properties of a fluid. This is a characteristic of many types of substances. Probably it is more exact to speak of these substances as plastic solids rather than as liquids; at any rate, this practice is followed by many recent authors. A plastic solid or an elastic sol owes its elasticity to the presence of structural elements which are often in the shape of fibers. The interlacing of fibrillar micellae provides a structure which offers resistance to distortion, and the result is a certain degree of elasticity. Under pressure, the structure tends to be broken down, and as the pressure is increased, there is an increase in fluidity, or in other words a decrease in viscosity. In the case of some colloidal solutions, mere shaking is sufficient to produce a marked change in fluidity. Thus vanadium pentoxide in concentrated colloidal solution is a pasty gel, when the gel is shaken it becomes quite fluid. Various other colloidal solutions behave in the same fashion, they are then said to be

thixotropic, and the phenomenon is called thixotropy (see FREUNDLICH '27).

When a colloidal solution has a definite form independent of a containing vessel, it is always elastic. If on the other hand, a colloidal solution is capable of flow, it may or may not have elastic properties. FREUNDLICH and SEIFRIZ\* describe a method for demonstrating the elasticity of colloidal solutions. A tiny sphere of some magnetic metal is suspended in the solution. It is then drawn to one side by a magnet. If the metallic particle springs back to its original position after the magnetic field is removed, this is certain evidence that the solution is elastic.

A more usual method of demonstrating the elasticity of colloidal solutions is to measure their elasticity at various rates of shear. True fluids have the same viscosity no matter what the rate of shear. On the other hand, elastic fluids (or plastic solids) have a lower viscosity as the rate of shear increases. Thus, to chose only a single example of many such observations, FREUNDLICH and ABRAMSON '27 in measuring the viscosity of an aging 1 % gelatin solution in a FLESS viscosimeter, found that the viscosity went through a whole series of diminishing values as the pressure on the solution was increased.

Concerning the elastic properties of protoplasm there is very little precise information. Probably every worker who has experimented with living animal cells knows that they regain their original form following distortion. Plant cells are usually surrounded by a stiff cell wall, so that this property is not so evident. But with animal cells, sea-urchin eggs for example, one could hardly escape knowing that the cells flatten under the pressure of a cover slip only to regain their shape again when the pressure is removed. This ability to resist distortion must depend in part on the surface tension of the film surrounding the egg, it must also depend on the elasticity of the membrane of the egg, which we have shown previously to be solid and therefore elastic (see p. 13). Whether the interior of the egg is elastic or not is a problem which requires further study than the mere observation of the elastic properties of the egg as a whole.

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\* FREUNDLICH and SEIFRIZ 1923, Zeitschr. f. physik. Chem., vol. 140, p. 233.

That cells are elastic, that is to say, that they regain their form following distortion has been known since the time of LEEUWENHOEK. Practically all the literature concerns blood cells. LEEUWENHOEK noted that red blood cells were frequently distorted when they passed through narrow vessels, but that they regained their original shape following distortion. This observation has been repeated by numerous subsequent workers. As early as 1822, J. C. SCHMIDT cites 13 authorities, all of whom had noted the elasticity of circulating red blood cells. To quote from SCHMIDT, "Daß die Blutkörper beim Durchgehen durch die kleinsten Gefäße oder Gänge gedrückt, und damit verlängert werden, daß sie aber nachher wieder ihre runde Gestalt annehmen. Dieses sahen und glauben LEEUWENHOEK, W. COWPER, Fr. W. HORCH, H. BAKER, H. MIHLES\*, J. KEIL, SENAC, FONTANA, Fr. B. ALBIN, L. CALDANI, G. REICHEL, SPALLANZANI, HUNTER, BLUMENBACH, POLI, J. DÖLLINGER". Most of these references are from the 18<sup>th</sup> century; for complete citations, see SCHMIDT's paper.

Following LEEUWENHOEK's observations, many authors experimented on the elasticity of red blood cells. Such experiments were not uncommon in the eighteenth century and in the early part of the nineteenth. The large corpuscles of salamander blood were soon recognized to be especially favorable material. In some experiments, the circulating blood of the salamander lung was compressed between glass or mica plates, and it was found that the size of the corpuscles might increase 3 or 4 or even 5 fold. Upon the removal of pressure, the corpuscles regained their original form. To quote SCHMIDT 1822 again, "Dellatorre will auch von dem Drucke zweyer Glimmerblättchen, zwischen welche man das Blut presst, die Gestalt der Blutkörper verändert, und nach aufgehobenem Drucke wieder hergestellt gesehen haben. Auch F. FONTANA will sie zwischen zwey Glimmerblättchen so zusammengedrückt haben, daß sie 4—5 mal breiter geworden seyen, nach aufgehobenem Drucke aber ihre wahre Gestalt wieder angenommen hätten." For later observations of the same sort, see ASCHERSON '38, WAGNER '38, ROLLETT '71. ROLLETT '71 also cites several other references on the same subject. NASSE '42 (see p. 93), and BOETTCHER '66 may also be consulted. STRICKER

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\* This is probably a reference to H. MILES.



'67 studied the elasticity of white blood cells and showed that they might be stretched to three or four times their original length.

In recent years there has been frequent reference to the elasticity of protoplasm, and it has often been stated that the living substance is highly elastic (see for example SEIFRIZ '26a). Actual data have been few. SEIFRIZ '26 b inserted microdissection needles into an amphibian red blood cell, and found that it could be stretched to three times its original length, and would then return to its original size if the tension were removed. SEIFRIZ is apparently unaware of the older literature in which the same observation was made without any possibility of an injury effect from the insertion of the needles into the cell. Granting that blood cells can be stretched and will then regain their original form, one does not know how much of this effect is due to surface tension, how much to the elastic properties of the cell membrane, and how much if any to the elastic properties of the interior protoplasm. SEIFRIZ himself is inclined to regard only the cell membrane as elastic (he calls it the cell wall). On the basis of some interesting reasoning, PONDER '24 had already pointed out that the elasticity of the red blood cell was due entirely to its membrane.

SCARTH '27 experimented with the protoplasmic strands of various plant cells. He states that he moved a needle back and forth in the axis of a strand of a *Tradescantia* cell. "The respective portions of the latter (i. e. the strand), lengthened or shortened elastically with little or no slip of the needle." Again "in *Spirogyra* the nucleus may be pushed from end to end of the cell and immediately recoils to its original position when released." These observations of SCARTH are of doubtful value. The mere fact that there is a strand in a cell visibly limited from the vacuole, is an indication that the strand must have a physical surface. The apparent elasticity of the strand as a whole might be due entirely to the surface tension of this surface. Concerning the actual structure of the protoplasmic strands of plant cells we know peculiarly little. Is the flowing protoplasm of these strands surrounded by a delicate tube-like membrane which prevents it from diffusing into the surrounding vacuole? Or do the protoplasmic granules move along a slender filament which occupies the center of the strand? Until we know more concerning the true

morphology of the protoplasmic strands of plant cells, it is rather difficult to interpret observations concerning their elasticity.

It is rather interesting to compare recent statements regarding elasticity of protoplasm with the statements of older workers. NAEGLI and SCHWENDENER '67 (see p. 403), after pointing out that when a *Spirogyra* cell is treated with a sugar solution it forms long threads between the cell body and the cell wall, state that if these threads are cut, the protoplasm of the thread is pulled back to the main mass of the cell. But they indicate clearly that this is not necessary evidence of elasticity. Apparently in 1867 one was well informed regarding surface tension phenomena.

From what has gone before, it must be obvious that the question of the elasticity or non-elasticity of the cell as a whole is an idle one. Anyone at all conversant with the properties of matter must realize that the surface tension of the cell in itself makes it resist distortion, and further that the presence of a rigid membrane such as is typical for animal cells likewise bestows elasticity on the cell as a whole. A much more important question is the problem of the elasticity of the protoplasm of the cell interior.

In the following discussion we shall consider first the possible elasticity of the protoplasm of the interior of plant and animal cells, and second the elasticity of the cell membrane of animal cells.

Very few workers have undertaken a measurement of the elasticity of the protoplasm which is contained in cells. In 1924, SEIFRIZ published a paper with the title "An elastic value of protoplasm", and this paper has often been quoted as indicating that the protoplasm of echinoderm eggs, and protoplasm in general, is elastic. In view of the fact that SEIFRIZ's paper constitutes one of the few attempts to measure the elasticity of protoplasm, his data will be considered in detail. SEIFRIZ cites only a single experiment. This was made with an *Echinarachnius* (sand dollar) egg. Into this egg, SEIFRIZ injected a nickel ball 16 microns in diameter. It has already been pointed out that such a procedure would be certain to produce severe injury (see p. 64), but for the purpose of discussion we will assume that the egg in question remained uninjured. The *Echinarachnius* egg measures 140  $\mu$ , and according to SEIFRIZ, there is a distinct cortical zone, whose

depth is "somewhat less than one-tenth of the diameter of the egg". Into this cortical zone, somewhat less than  $14\ \mu$  in thickness, the  $16\ \mu$  nickel particle was imbedded. From these figures it is quite obvious that the particle was in direct contact with the outer membrane of the egg. When the current of an electromagnet was turned on, and the egg was held by two needles, the nickel particle moved approximately  $9\ \mu$ . SEIFRIZ does not definitely state whether or not the nickel particle returned to its original position after the magnetic field was removed. Apparently it did return, for he made no correction in his "stretching value" for such a failure to return. How it could have failed to return is difficult to understand. When the nickel particle moved, it must have stretched the egg membrane, and the elasticity of this membrane was certainly sufficient to restore the nickel particle to its original position. It is thus obvious that SEIFRIZ's experiment gives us no real information regarding the elasticity of the interior protoplasm of the *Echinarachnius* egg. We do not even know whether or not this protoplasm is elastic. In the same paper in which he makes the elasticity test, in a short section on viscosity SEIFRIZ describes one or two tests in which a nickel ball moved through the center of the egg, and apparently did not return after the magnetic field was removed. This may indicate either absence of elasticity or adhesion to the outer cortex or membrane of the egg.

An elastic fluid is a fluid with an internal structure. If it is subjected to pressure or distortion, the structure tends to break down and this is reflected in a more or less pronounced decrease in viscosity. Here then is a means of determining whether or not the interior protoplasm of living cells is elastic.

Some very interesting observations are recorded in a paper of HEILBRONN's ('22). It will be remembered that this worker introduced tiny iron rods into the plasmodia of various myxomycetes. These rods were then made to rotate as a result of their being exposed to a magnetic field from an electromagnet. The strength of the magnetic field, as determined by the amperage of the current which passed through it, was then taken as a measure of the viscosity. It is interesting that HEILBRONN never makes any mention of the return of the iron rods to their original position, and from his method of describing his experiments it is quite certain that no such return occurred. It is thus apparent

that the interior protoplasm of slime mold plasmodia is not highly elastic. But the most important experiments of HEILBRONN, from the standpoint of the elasticity question, are the experiments in which he repeatedly twisted the iron rods in the attempt to discover whether such repeated twistings had an effect on the viscosity of the protoplasm. These experiments we shall now proceed to consider.

The results obtained by HEILBRONN were of two sorts. In the first place, he found that for *Reticularia*, which has relatively large plasmodia, the viscosity showed no decrease following frequent twistings of an iron rod. The experiment was carried out in the following way. An iron rod within a *Reticularia* plasmodium was placed at right angles to the lines of force of the magnetic field. The number of amperes current necessary to turn it parallel to the lines of force, was measured. Then the stage of the microscope was turned through  $90^{\circ}$ , and the measurement repeated. In the only experiment cited by HEILBRONN, the measurements of viscosity were repeated at minute intervals. It was found that the viscosity, as determined by the amperage of the current passed through the electromagnet, remained constant for 18 successive determinations at a value of 3.5 amperes. Then the viscosity increased to a value of 3.6 amperes and remained at this value for 7 more determinations, following which the experiment was discontinued. It is thus apparent that the movement of an iron rod through the protoplasm of *Reticularia* plasmodia does not produce a viscosity decrease. This is strong evidence that the protoplasm of this type of plasmodium is inelastic.

On the other hand, when the smaller plasmodia of *Badhamia utricularis* and *Physarium cinereum* were studied, the viscosity was found to drop following frequent determinations. In the case of *Badhamia utricularis*, viscosity determinations could only be made at intervals of several minutes. The plasmodium is so small that the iron rod had to be oriented with a needle following each measurement, in order to make certain that it had room to turn. HEILBRONN is of the opinion that the insertion of the needle may very well have had an effect on the viscosity value, and he cites an experiment in support of this view. Following 12 successive determinations on a *Badhamia* plasmodium, the viscosity value in amperes was found to drop from 1.9 to 1.4. *Physarium* plasmodia resemble those of *Badhamia* in that there is barely

room enough for the iron rod to turn. In this form also, HEILBRONN found the viscosity to decrease following successive measurements. Thus after 11 successive measurements, the viscosity value was 1.9 as compared with an initial value of 2.2. Nine subsequent measurements resulted in no further decrease in viscosity.

There are two possible interpretations of these experiments of HEILBRONN's. At first sight we would conclude that the protoplasm of *Reticularia* is inelastic, whereas that of *Badhamia* and *Physarium* is elastic. But it must be remembered that although the plasmodia of *Reticularia* are large, those of *Badhamia* and *Physarium* are small. In the latter two forms, the turning of the iron rod tends to be prevented by the presence of the ectoplasm. As has been shown previously (see p. 64), the ectoplasm of myxomycete plasmodia is certainly a gel, and it is doubtless elastic. Hence in *Badhamia* and *Physarium* the observed elastic effect is due not to the internal protoplasm, but to the outer ectoplasm. If we accept this second interpretation, we are no longer faced with the necessity of assuming that two closely similar types of living substance differ in a fundamental property.

We conclude therefore, that as far as the evidence goes, the internal protoplasm of slime mold plasmodia is inelastic.

There is an interesting experiment of RHUMBLER '02, '14, which should be cited in this connection. RHUMBLER studied the effect of pressure on the speed of rotation of *Chara* protoplasm. The cells of the alga *Chara* were mounted on a slide, and with the aid of a special apparatus, pressure was applied to the cover slip from above. Pressures up to 4.6 atmospheres had no effect on the rate of protoplasmic streaming, the protoplasm flowed neither more slowly nor more rapidly. A similar result had, as a matter of fact, previously been obtained by HÖRMANN '98. This author subjected cells of *Nitella* in a glass tube to a pressure of two atmospheres. In these experiments, the tube containing the cells was connected with a manometer tube filled with mercury. HÖRMANN found that a pressure of two atmospheres had no effect on the rate of streaming (see also LAUTERBACH '21). At first sight, it would seem that the experiments of HÖRMANN and RHUMBLER offer proof of the inelasticity of the streaming protoplasm. For if the protoplasm were an elastic fluid (or a plastic solid), we would expect a decrease in viscosity and an

increase in the rate of flow with increase in pressure. But it should be pointed out that the pressure applied to an entire cell of *Chara* or *Nitella* is hardly comparable to the pressure which is applied to gelatine as it is forced to flow through a capillary tube. We are probably not justified, therefore, in regarding the HÖRMANN-RHUMBLER experiment as a demonstration of the inelasticity of the protoplasm of the cells of *Chara* or *Nitella*.

As mentioned previously, a standard method of determining whether or not a fluid is elastic, is to make viscosity measurements at varying rates of shear. If the viscosity remains constant, the fluid is inelastic. Thus, for example, if we measure the rate of flow through a capillary tube, in the case of an inelastic or true fluid, the speed is proportional to the pressure, the liquid obeys POISEUILLE's law for the speed of flow of liquids through tubes, and the viscosity remains constant as the pressure is varied. On the other hand, if the material we are studying is elastic, there is no longer a strict proportionality between the speed of flow and the pressure, for the viscosity becomes less and less as the pressure is increased. In general, no matter what type of viscosity measurement is employed, for elastic materials the viscosity becomes less as the shearing force increases, whereas for inelastic materials the viscosity remains constant as the shearing force changes.

In using the centrifugal method to measure protoplasmic viscosity, it is a simple matter to vary the shearing force. One needs only turn the centrifuge at different rates of speed. The centrifugal force varies as the square of the number of turns per second.

In a series of tests on the protoplasm of *Cumingia* eggs (HEILBRUNN '26a), the centrifuge handle of a small hand centrifuge was turned once per second, once every two seconds, once every three seconds, and once every four seconds. The speed of movement of the protoplasmic granules was determined for each of these rates of turning. In the *Cumingia* egg, the fat granules move a little more rapidly than do the pigment granules. In the following table (Table I.), the time in seconds necessary for the fat granules to move far enough so as to give the appearance of a definite zone at one end of the egg is noted in the second column, under *T*. The time in seconds necessary for the pigment granules to move far enough so as to give the appearance of a

definite zone is shown in the fourth column, under  $T'$ . In the first column, under  $S$ , are shown the number of seconds required for a single turn of the centrifuge handle. If the protoplasmic fluid through which the granules move is a true fluid, their speed of movement should vary directly with the centrifugal force, that is to say, with the square of the number of turns the centrifuge tubes make per second. From any one value for granule speed, it is easy to calculate what the speed would be for other amounts of centrifugal force, on the assumption that the granules really do move through a true fluid. In the table, the calculated values for  $T$  and  $T'$  were obtained from the second series of values. The calculated values for  $T$  and  $T'$  are given in the third and fifth columns respectively.

Table I

$S$	$T$ observed	$T$ calculated	$T'$ observed	$T'$ calculated
1	2	3	3	$3\frac{1}{2}$
2	12	—	14	—
3	27	27	33	$31\frac{1}{2}$
4	48	48	56	56

From the table it is easy to see that the observed speeds are close to the speeds calculated on the assumption that the protoplasm through which the granules travel is a true fluid. The agreement is particularly satisfactory for the last two values in each case. When the centrifuge is turned at top speed, at the rate of one turn of the handle in one second, the observed value for the time of granular movement is too low. This is readily understandable from the conditions of the experiment. When the centrifuge handle is turned fast, it is not possible to stop it immediately following the expiration of a given number of turns. Thus when the handle is turned two times in two seconds, it continues to move for a good fraction of a second before it can be stopped. The percentage of such error is also greater when the total number of turns is small.

The data in Table I are perhaps more readily understandable if one calculates the protoplasmic viscosity for different amounts of centrifugal force. This has been done in Table II., in which only the data in the second half of Table I. have been utilized.

The viscosity values in Table II are arbitrary relative values, which however happen to be close to the actual values in terms of the viscosity of water.

Table II

Centrifugal force in terms of gravity	Viscosity of <i>Cumingia</i> egg protoplasm in arbitrary units
310.5	3.5
552	3.7
1242	3.5
4968	3

Obviously, the viscosity remains constant as the centrifugal force varies. As pointed out previously, the value obtained with very high centrifugal force is not accurate, for it is impossible to stop the centrifuge quickly enough. Such an error would tend to make the viscosity value too low, and this is apparently the explanation of the low value obtained with a force 4968 times gravity. Attention should be called to the fact that the variation of shearing force in the above tests is greater than that usually found in similar experiments of colloid chemists on inanimate material. It should also be noted that the shearing force is not excessively great, for the speed of the moving granules is really very slow. Thus at slower centrifugal speeds the granules only travel 15 or 20 microns in a minute.

The data that we have presented are not very extensive. As far as they go, they show that the protoplasm of the *Cumingia* egg is not elastic. Other unpublished results tend to bear out this conclusion, not only for the *Cumingia* egg, but for the *Arbacia* egg as well.

From the preceding discussion it is apparent that in spite of the various papers that have been written on the elasticity of protoplasm, there are in the entire literature only two experiments which really give any information concerning the elasticity of the internal protoplasm of cells. At any rate only two such experiments have been discovered in a search of the literature. HEILBRONN's experiment in *Reticularia* plasmodia shows the internal protoplasm in this species to be inelastic. The centrifuge



experiment of HEILBRUNN also indicates an inelasticity of the protoplasm of the *Cumingia* egg.

One would hardly be able to conclude much from these two experiments, if it were not for the fact that there is some excellent negative evidence which supports the view that undifferentiated protoplasm is typically inelastic. When elastic materials are subjected to a one-sided pressure, they are always doubly-refractive. Now in spite of the fact that many observers have studied cells under crossed nicols, no one has ever described any type of undifferentiated protoplasm which is doubly refractive. When a biologist studies cells, he usually subjects them to the pressure of a cover slip, and as the preparation dries, this pressure may be considerable. The conditions of observation would therefore be favorable for the demonstration of anisotropy, if it were present. As a matter of fact, the cell membranes can be seen to be doubly-refractive. But in spite of the fact that the polarization microscope was in very common use in the middle of the last century, there is apparently no record of any description of undifferentiated protoplasm as doubly-refractive. VALENTIN '61 states specifically that the protoplasm of various egg cells is isotropic. And DIPPEL '72 (part 2, p. 324) states categorically, "Sämtliche nur aus Protoplasma aufgebaute nicht krystallisirte Inhaltskörper (ohne Stärkeeinschluss) modificiren das polarisirte Licht in keiner Weise und geben sich somit als einfach brechende zu erkennen".

An especially interesting observation is that of VALENTIN '64. He studied streaming plant protoplasm under a polarization microscope. From his careful description of the anisotropy of an occasional crystal, and his failure to remark on the anisotropy of any other elements, it seems certain that the main mass of the flowing protoplasm was isotropic. An elastic fluid could never appear as isotropic when it was in a state of flow.

It must not be thought that all protoplasm in cell interiors is inelastic. On the contrary, there are certain indications that in many cells there are at least elastic elements in the protoplasm. We have already had occasion to refer to the presence of definite fibrillar elements in some types of cells (see p. 17 and p. 80). Such fibrils are beyond any question elastic, as the very fact that they possess form indicates. Even in marine eggs, in which the main mass of the protoplasm is inelastic, elastic fibers appear

during cell division. When unfertilized *Cumingia* eggs are centrifuged, although most of the protoplasmic granules move through the cell readily enough, here and there occasional granules can be seen to be entangled in the astral rays of the mitotic spindle of the first maturation division (cf. HEILBRUNN '26c, p. 271). These astral rays are surely elastic. This is very evident in the *Arbacia* egg, in which the astral radiations can be seen to exert a pull or tension on the enclosing membrane of the cell to which they are attached. This membrane, the so-called hyaline layer, is at first perfectly smooth in contour. But with the appearance of the amphiaster it becomes indented, so that it is often very obviously crenate (see HEILBRUNN '20a, p. 222). Almost certainly these indentations are the result of a pull of the astral ray fibers, and so HEILBRUNN interpreted them. The uneven appearance of the egg contour becomes exaggerated when the eggs are centrifuged. Then the astral ray fibers exert a still stronger pull on the periphery of the cell, so that the cell is often very much distorted.

The astral ray fibers are gels, and they are apparently thixotropic gels. This follows from the fact that shaking results in a disappearance of the astral radiations. WILSON '01 shook sea-urchin eggs violently just as they began to divide. The division was suppressed, and the astral rays disappeared (see WILSON's Fig. 58). BOVERI '97 had previously found that the division of the sea-urchin egg could be prevented by pressure. SEIFRIZ '24a also describes a disappearance of the mitotic spindle when eggs of *Echinarachnius* are subjected to pressure.

There can be no question but that the visible fibrils or fibers contained in protoplasm or forming a part of protoplasm are elastic. Such a statement hardly requires proof. It also seems probable that the dispersion medium of protoplasm, that is to say the medium in which granules and fibrils are suspended, is without elasticity in many if not in all types of cells. This follows both from the experimental evidence of HEILBRUNN and HEILBRUNN, and from the fact that undifferentiated protoplasm is not doubly refractive.

Further tests of the elasticity of protoplasm seem desirable. Whenever a cell is capable of having its viscosity tested by the centrifuge method, it is possible to decide whether or not the protoplasm is elastic. One has only to make a series of tests at

different centrifugal speeds. If in these tests the viscosity remains constant, then it seems certain that the protoplasm is inelastic.

The second phase of our subject deals with the elasticity of the cell membranes of animal cells. These membranes are certainly rigid, and there is thus no doubt but that they are elastic. It therefore becomes of interest to attempt a measurement of this elasticity.

With the aid of a horizontal microscope VLÈS '26b studied the deformation or flattening of sea-urchin eggs when they came to rest on a flat surface. In VLÈS' terminology, if the horizontal diameter of the eggs is  $a$ , and the vertical diameter by  $b$ , then the eccentricity of the eggs can be expressed by the fraction  $\frac{a-b}{b}$ .

For normal eggs surrounded by jelly, VLÈS found this eccentricity to average 0.12, when the eggs came to rest on a glass plate. If the jelly was washed off with 3 % KCN, the eccentricity was found to be 0.026\*. From these values, VLÈS calculates the surface forces of the egg on the basis of an empirical formula which he obtains from an experimental study of small droplets of various liquids. According to VLÈS, the surface forces include the following: "les forces de tension superficielle (au sens capillaire du mot), forces interfaciales exercées à la limite entre les liquides extérieurs et les substances de phases différentes constituant la périphérie de l'œuf; éventuellement, les forces élastiques développées par des membranes enveloppantes; et enfin et surtout les composantes superficielles des forces de rigidité des gels intérieurs, cisailées par toute déformation mécanique dont l'œuf peut être le siège". From the previous discussion, it is apparent that the last mentioned forces do not exist. The surface forces of VLÈS can then be taken to include surface tension forces and forces due to the elasticity of the egg membrane. For these combined forces, VLÈS calculates a total force of 10 to 25 dynes per centimeter.

There is also another possible way in which one can study the elastic and surface tension forces of the membrane of a cell. When a spherical cell, such as for example a sea-urchin egg, is placed in a hypotonic solution, the cell does not enlarge to the

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\* It seems probable that 3% KCN is toxic. A simpler procedure would have been to shake the eggs free from jelly, or to centrifuge them.

volume it would occupy if no elastic membrane were present. Thus if *Arbacia* eggs are placed in a solution of 75 % sea-water and 25 % distilled water, we would expect that the volume of the cell would increase until the concentrations of electrolytes inside and outside were the same. In other words, we would expect the cell to increase in volume until the final volume would be to the original volume as 75 is to 25, or 4 to 3. Actually when a sea-urchin egg is placed in 75 % sea-water, it does not become 1.33 times as large, but only 1.28 times as large as it was before exposure to the hypotonic solution (unpublished experiments)\*. The difference is due both to the elastic properties of the membrane and to surface tension forces. It is interesting to note that when a *Cumingia* egg is placed in a 75 % solution of sea-water, it increases to only 1.25 times its original volume. This lesser increase is to be associated with the greater thickness and rigidity of the membrane surrounding the *Cumingia* egg.

In a study of the volume changes of spherical cells in hypotonic solutions, it is also possible, to some extent at least, to separate the effects due to surface tension and those due to elasticity or rigidity of the membrane. When a surface tension film is stretched, the force which tends to diminish its surface suffers no increase. On the other hand, when a rigid membrane is stretched, the force tending to decrease the surface becomes ever larger. If we place cells like sea-urchin eggs in a series of hypotonic solutions of decreasing strength, as the cells increase more and more in volume there is no change in the surface tension force tending to prevent enlargement of the cell, but there is a very pronounced change in the force due to the elasticity of the rigid membrane surrounding the cell. When *Arbacia* cells are placed in 50 % sea-water, the increase in volume of the egg is very much less than we would expect if the egg were free from the constraint of a surrounding membrane. The surface tension effect is no greater for the 50 % solution than for the 75 % solution.

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\* In making measurements of increase in volume of eggs in hypotonic solutions, too great a time must not be allowed to elapse before the measurement is made. As water enters the egg, it produces changes in the protoplasm which probably result in the freeing of electrolytes. It will be remembered that the sea-urchin egg contains a large excess of bound electrolytes (see p. 25).

Hence in proportion to the greatly increased pressure of the elastically stretched membrane, the surface tension force becomes negligible. We are thus in a position to make a direct study of the elastic properties of the rigid membrane surrounding the sea-urchin egg.

We might for example study the changes in elasticity which the membrane undergoes when it is placed in contact with various reagents, provided that these reagents have no very great effect on the permeability and hence on the osmotic forces acting on the membrane. Or it might be possible to determine the modulus of elasticity of the membrane. It is not a very difficult matter to derive an equation for the case of an elastic membrane stretched by a pressure pushing it out from within. Such an equation has indeed been published by FRANK '10, and if one is willing to make certain assumptions regarding the osmotic forces acting on the membrane, one might perhaps use the equation to calculate absolute values for the modulus of elasticity.

It must not be forgotten that as the volume of the cell increases, there is probably a release of some of the bound electrolytes of the protoplasm. We have already seen that these are present in abundance (see p. 25). If bound electrolytes are set free as the cell enlarges, they would tend to make the cell expand even further and this would have the effect of making the elasticity values too low. There is also a difficulty owing to the presence of granules within the protoplasm. It is possible that the granular volume does not increase at all when the eggs are placed in hypotonic solutions. If this were the case it would in itself account for the fact that sea-urchin eggs increase to only 1.28 times their original volume in 75% sea-water, but it would not account for the results obtained with 50% sea-water. The granular volume of eggs in different solutions can be roughly measured in centrifuged eggs, so that the influence of changes in granular volume can be determined.

It is thus probable that in spite of difficulties of one sort or another, experiments with hypotonic solutions may furnish valuable information regarding the elastic properties of the cell membrane. Further investigation is therefore likely to yield results of considerable interest.

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## CHAPTER VII

# THE ACTION OF TEMPERATURE

For the most part, life is only possible within very narrow limits of heat and cold. The natural temperatures of our planet do not cover a very wide range, and yet many plants and animals are quite unable to withstand the extremes of the temperatures they are subjected to. Both in the tropics and in the arctic regions, living organisms are very frequently killed by the heat or the cold of their ordinary environment, and even in the temperate zone, such heat or cold death is by no means uncommon. In many instances the distribution of animals and plants is governed by their ability to withstand high or low temperatures. A priori, it seems very likely that the action of heat and cold on protoplasm finds expression in colloidal changes. Experiment shows this prediction to be verified.

But the action of temperature is not entirely lethal. Between the limits of heat and cold in which protoplasm can remain alive, there is a range of temperatures which can produce marked effects on the life and activity of the cell. Some of these effects are doubtless due to a direct influence of the temperature on the rate of chemical reaction within the cell. Many authors have stressed this aspect of the subject, and some of them have completely neglected the physical effects which temperature change might have. From the standpoint of physical chemistry, it is evident that the rate of chemical reaction in a heterogeneous system is dependent on the physical properties of the various phases of the system, especially on the degree of dispersion, if the system be a colloidal one. It is thus of interest to inquire whether the ordinary temperatures of the environment can produce recognizable changes in the physical properties of protoplasm, and whether such changes if observable can be correlated with known diffe-

rences in the behavior of protoplasm at one temperature or another.

There is however a more direct interest in the study of the action of temperature on protoplasm. The physical changes which occur in protoplasm as the temperature is raised or lowered may very well throw light on some of the fundamental properties of the living colloid.

Of all the independent variables of the cell, temperature is perhaps easiest to control. If we change the chemical constituents of the environment, we have no certain knowledge of how far such a change is passed on to the cell interior. But when we place cells at a given temperature, we can feel certain that we know the temperature of the interior protoplasm.

In discussing the action of temperature, we shall consider the following topics:

1. The changes produced by moderate temperatures.
2. The heat death of the cell.
3. Death due to low temperatures.

Most fluids undergo a progressive decrease in viscosity as the temperature is raised. That such a decrease in viscosity also occurs in protoplasm is indicated by the results of FAURÉ-FREMIET '13. He measured the viscosity of the protoplasm of *Ascaris megalocephala* eggs by noting the length of time necessary for the displacement of mitochondria granules when the eggs were centrifuged. FAURÉ-FREMIET's results are given in the following table. In this table the second column gives the time necessary to throw the mitochondria from one end of the cell to the other. FAURÉ-FREMIET used a centrifugal speed of 2500 turns per minute, but the radius of turn is not given in his description of his experiments.

Table I. Viscosity of *Ascaris* egg protoplasm at different temperatures according to FAURÉ-FREMIET '13

Temperature	Time (i.e. relative viscosity)
35°	4 min.
30°	8 "
23°	20 "
18°	45 "
8°	225 "

FAURÉ-FREMIET's data show that the viscosity of *Ascaris* egg protoplasm is decreased four or five fold by a rise in temperature of ten degrees. An older observation of HOGUE '10 also indicates a decrease in the viscosity of *Ascaris* egg protoplasm as the temperature is raised. She found that the Brownian movement of granules in centrifuged *Ascaris* eggs is more rapid at higher temperatures.

It should be noted that the optimal temperature for the development of *Ascaris megalocephala* eggs is 37° (see BONFIG '25). Thus the temperatures studied by FAURÉ-FREMIET are all below that optimal or normal for development. If one compares the behavior of various types of protoplasm in relation to temperature, one often forgets that the optimal temperature is very different for different organisms. A temperature of 37° is normal for *Ascaris*, but sea-urchin eggs exposed to this temperature are killed in a minute or two.

For the eggs of the annelid *Nereis diversicolor*, PANTIN '24 also found that the viscosity of the protoplasm decreased as the temperature was raised. PANTIN determined the viscosity by centrifuging the eggs. He made measurements at only four temperatures. The results are shown in the following table, in which the viscosity at 10° is taken as unity.

Table II. Viscosity of protoplasm of eggs of *Nereis diversicolor* at various temperatures (PANTIN '24)

Temperature	Relative viscosity
— 0.7°	1.95
10.0°	1.00
20.0°	0.71
30.0°	0.57

PANTIN's results agree very well with the older results of F. and G. WEBER '17 for plant cells. These authors measured the viscosity of the starch sheath cells of the stem of *Phaseolus multiflorus* (a leguminous plant) at different temperatures. The viscosity was determined by measuring the speed of fall of starch grains through cells in sectioned slices of the stem. Apparently the experimental error was not great, for when a series of tests was made, the same value for the viscosity was always obtained.



It made no difference whether the protoplasm was heated to a given temperature or cooled to a given temperature, the viscosity value remained the same. This is an important point and one not considered by other authors who have studied the effects of temperature on protoplasmic viscosity. The results of F. and G. WEBER are given below in tabular form. The time of fall is in seconds and is a measure of the viscosity. Comparisons of viscosity at various temperatures should only be made for a given cell, each row of figures represents the data for such a cell.

Table III. Starch sheath cells—*Phaseolus* (F. and G. WEBER)  
Temperature

0°	4°	8°	10°	14°	20°	24°	30°	34°	40°	44°	50°	54°	60°
—	—	—	8	—	6	—	—	—	—	—	—	—	—
—	—	—	14	—	11.5	—	10	—	—	—	—	—	—
—	—	—	—	—	—	—	14	—	11	—	8	—	7
—	—	—	—	—	—	—	6	—	5	—	4	—	3.5
—	—	—	—	20	—	16	—	10	—	7	—	—	—
—	16	14	12	11	8	—	4	—	—	—	—	—	—
—	—	—	20	—	12	—	7	6	—	—	—	—	—
—	—	—	32	—	18	—	14	—	10	—	9	—	—
—	—	—	—	—	13	—	10	—	7	—	6	—	—
21	—	—	15	—	11	—	8	—	—	—	—	—	—
—	—	—	12	—	9	—	7	—	5	—	—	—	—
20	—	—	15	—	10	—	7.5	—	6	—	—	—	—
—	9	7	6.5	5	—	—	—	—	—	—	—	—	—
—	—	11	10	—	8	—	7	—	6	—	5	—	4.5
—	—	—	8.5	—	7	—	6	—	5	—	—	—	—
—	—	16	—	14	—	12	—	9.5	—	8	—	6.5	—

Average  $Q_{10}$

1.51 | 1.41 | 1.37 | 1.27 | 1.20 | 1.14

The last row of figures in the above table gives the  $Q_{10}$ , that is to say, the ratio of the viscosity at a given temperature to the viscosity at a temperature ten degrees higher. The values for this temperature coefficient decrease for higher temperatures. This is typical for water and watery solutions, and the WEBERS point out that the values for  $Q_{10}$  obtained by them are very similar to the  $Q_{10}$  values which have been found for protein solutions and for blood plasma.

In a later paper, WEBER and a co-worker made another study of the effect of temperature on the protoplasmic viscosity of the starch sheath cells of *Phaseolus multiflorus* (WEBER and HOHENEGGER '23). In this work they studied only the action of low temperatures. In order to avoid any possible pathological effects due to sectioning, the stems were centrifuged whole, and were then sectioned just prior to examination. It was found that when the temperature was lowered to  $-2^{\circ}$ , the viscosity increased at least fourfold and probably more. This viscosity increase occurred after an exposure of only a few minutes (5 minutes in one experiment). When the plants were again brought to room temperature, the viscosity returned to its original value, so that the effect of cold was reversible. WEBER and HOHENEGGER also used temperatures somewhat higher than  $-2^{\circ}$ . The highest temperature for which they give data is one of  $+5^{\circ}$ . For this temperature they record a viscosity increase, as compared with room temperature, of at least three fold.

It is clear that the viscosity increases described by WEBER and HOHENEGGER are somewhat greater than those previously found to occur in the cold by F. and G. WEBER (see Table III.). Thus in two experiments, F. and G. WEBER found the viscosity at  $0^{\circ}$  approximately twice as great as that at  $20^{\circ}$ , whereas WEBER and HOHENEGGER found the viscosity to increase at least fourfold when the plants were cooled to about  $0^{\circ}$ . Perhaps, as WEBER and HOHENEGGER suggest, there may be an error involved in sectioning the plants, as was done by F. and G. WEBER (see also p. 43).

FAURÉ-FREMIET, F. and G. WEBER, and PANTIN have all obtained results which indicate that the protoplasmic viscosity in the cells studied by them decreases regularly as the temperature is raised. That this is not the case in all cells was shown by HEILBRUNN '20. He found that in *Arbacia* eggs the viscosity was lower in the neighborhood of the freezing point than at room temperatures. In his description of his experiments, HEILBRUNN records the temperature as  $-3^{\circ}$ . Probably it was not as low as this, for he measured the temperature of the freezing mixture surrounding the tube containing the eggs, and not the temperature of the fluid immediately in contact with the eggs. This may involve an error, and from subsequent tests it seems certain that the temperature of HEILBRUNN's experiments was not as low as  $-3^{\circ}$ .

In 1922, with the aid of his magnetic method, HEILBRONN determined the viscosity of the protoplasm of the slime mold *Reticularia* at different temperatures. HEILBRONN's data appear to be very accurate, but his figures are given in terms of a relative measurement which varies from experiment to experiment. For the convenience of the reader, HEILBRONN's figures have been recalculated, and in the following table they are given in percents of the value at 16°—17.5° C.

Table IV. Slime mold plasmodia on glass (HEILBRONN '22)

Temperature	Relative viscosity
12°	93.3%
16—17.5°	100 %
26°	84.3%
33°	72.2%
35°	Variable, from 63.3 to 214%
40°	More than 333%, no longer measurable

In Table IV., the value at 12° is the average of three tests, that at 26°, the average of five tests. HEILBRONN interprets the variable value at 35° as indicating that the protoplasm is injured and has become separated into phases of high and low viscosity. If the measurements were made in different regions, as HEILBRONN's explanation indicates, it seems just as logical to assume that some parts of the protoplasm coagulate more quickly than others. Whether we accept this interpretation or not, it is obvious that the viscosity of the protoplasm drops to a minimum value before coagulation occurs. This is apparently typical for various types of protoplasm. In Table IV. it is to be particularly noted that the viscosity of the protoplasm goes through a maximum at 16—17.5°, and it is lower both above and below this temperature.

The measurements of HEILBRONN recorded in Table IV. were made on slime mold plasmodia which were resting directly on a glass surface. As pointed out previously (see p. 62), the viscosity of slime mold protoplasm is less when the plasmodia are placed on moist filter paper. For such slime molds on moist filter paper, the viscosity varies hardly at all for different tempe-

ratures. Thus HEILBRONN placed pieces of *Reticularia* on moist filter paper and warmed them to various temperatures. After an hour in contact with moisture at a given temperature, the viscosity was measured with the magnetic method. The results are given in the following table, in which the original scale of HEILBRONN is retained.

Table V.

Slime mold plasmodia on moist filter paper (HEILBRONN '22)

Viscosity on glass at room temperature	Temperature of the experiment	Viscosity on moist filter paper after an hour
3.7	12°	3.0
3.3	12°	2.9
3.5	17°	2.4
3.5	17°	2.5
3.5	17°	2.5
3.3	27°	2.5
3.6	27°	2.4
3.5	27°	2.5
3.6	30°	2.4
3.6	30°	2.4

Within the limits of experimental error, the viscosity of the plasmodia saturated with water remains constant from 17° to 30°. Apparently below 17° there is an increase in viscosity, instead of a decrease as previously recorded for plasmodia on glass. But there is also another possible interpretation of this lower viscosity at 12°. When plasmodia are placed on moist filter paper, their viscosity decreases slowly. In the only three experiments cited by HEILBRONN, the time required for the viscosity to reach a constant low value was 30 minutes in one case, and an hour, and an hour and ten minutes in the other two cases. It seems likely that at lower temperatures a longer time is required for equilibrium. In view of the fact that the above experiments were all made at the expiration of an hour, it is quite possible that at 12° enough time had not elapsed for the protoplasmic viscosity to have reached its final minimal value. The fact that the viscosity remains constant between 17° and 30° is surely difficult to understand.

It has been shown that for *Arbacia* eggs and for *Reticularia* plasmodia on glass the viscosity may decrease as the temperature

is lowered. The same phenomenon occurs in the egg of the clam *Cumingia*. For the protoplasm of this egg, HEILBRUNN '24 was able to plot a fairly complete temperature-viscosity curve. This

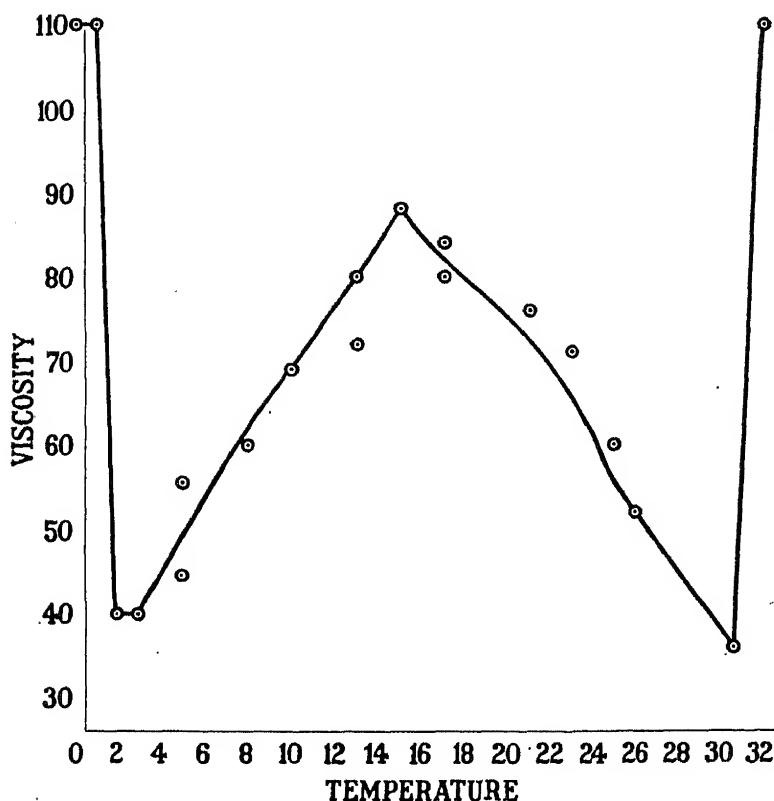


Fig. 3. Temperature — viscosity curve (HEILBRUNN '24a).

curve is shown in Fig. 3. The abscissae are degrees Centigrade, the ordinates relative viscosity units. The viscosity was determined by centrifuging the eggs. In order to obtain greater precision, the eggs were centrifuged relatively slowly. The centrifugal force used was 311 times gravity. The ordinates in the

curve represent the number of seconds required to form the zones typical for the *Cumingia* egg, (see Fig. 1, p. 45). For each point on the curve a number of tests had to be made, for of course the eggs could not be examined while they were being centrifuged. "Thus at 23° it was shown that 17 turns of the centrifuge (for 68 seconds) did not produce zones in the egg, whereas 18 turns (72 seconds) did produce zones. The viscosity was therefore given as 72, for the limits of accuracy of the method did not make it advisable to differentiate any closer than this." The centrifuging was done at room temperatures, and this introduces an error, especially when the viscosity is high, and when the temperature of the experiment differs markedly from that of the room. For this reason the determinations at 0° and 31° are not to be regarded as very exact. They are rather to be thought of as minima, although other experiments at higher centrifugal speeds showed that the tests at the low temperature are not very far wrong.

The viscosity of the protoplasm of the *Cumingia* egg goes through a maximum at about 15°. This is essentially the same result that was obtained by HEILBRONN for slime mold plasmodia on glass. It is noteworthy also that in addition to the sharp viscosity increase that occurs at 31°, there is also a sudden viscosity increase at 1° above the freezing point. In both cases, when either the temperature is raised or lowered, there is a minimum in viscosity just before the marked increase in viscosity takes place.

From what has gone before, it seems apparent that there are two types of temperature-viscosity curves which may be obtained for protoplasm. Some cells show a maximum viscosity at around 15°, whereas in other cells the viscosity decreases progressively as the temperature is raised. That there are really two types of curves is not absolutely certain, for FAURÉ-FREMIET's results were all obtained at temperatures below the optimum, PANTIN's data for the *Nereis* egg are too few, his four measurements might almost be superimposed on the curve for the *Cumingia* egg, and finally the WEBERS worked with sectioned tissues and their results were not exactly confirmed by WEBER and HOHENEGGER on intact plants of the same species. Nevertheless, it does seem likely that the behavior of protoplasm toward temperature does show differences for different types of material.

The protoplasm of the egg of *Nereis* is highly viscous (at any rate this is true for the form of *Nereis* found at Woods Hole), whereas the protoplasm of the *Cumingia* egg is relatively very fluid. One would hardly expect them to show the same behavior toward temperature change.

It might be expected that if some types of protoplasm show a maximum in viscosity at 15°, that this would be reflected in the relation which some biological processes show to temperature. There are at least several examples in the literature in which processes of one sort or another have shown a maximum or minimum at around 15°. Thus for example PLOUGH '17 found a maximum in crossingover in *Drosophila* at 13°, VAN DILLEWIJN and JACOB '24 found a maximal response of the pedal muscles of the snail *Helix* at 21°, and PEREIRA '24 describes a maximum in the irritability of frog muscle at about the same temperature. Moreover it has been known for a long time that the temperature coefficient of various biological processes may go through a sharp change at about 15°. Many instances of such a change are recorded in KANITZ '15 (see also CROZIER '24).

Numerous authors have studied the effect of temperature on protoplasmic streaming. Generally the rate of flow decreases steadily as the temperature is raised. NAEGELI found the increase in speed to follow a geometrical progression, that is to say a logarithmic curve is obtained if his results are plotted. On the other hand VELTEN '76a found an arithmetic progression and his results when plotted fall along a straight line. Later investigators have tended to confirm VELTEN, rather than NAEGELI. Fig. 4 shows the curve plotted by LAMBERS '25 for a cell of *Nitella mucronata*. The curve is particularly interesting, because it shows that the order of the observations, as indicated by the numbers on the curve, makes no difference. That is to say, the same value is obtained when the protoplasm is heated or cooled to a given temperature. In no case is it possible to say definitely that increased speed of protoplasmic streaming is due solely to viscosity change, but the results of LAMBERS suggest that the protoplasm of *Nitella* undergoes a progressive decrease in viscosity as the temperature is raised. This is the view favored by LAMBERS. EWART '03 (p. 61) thinks that both viscosity change and a change in the motive force play a role in the increased speed of streaming at higher temperatures. He points out that whereas viscosity

typically decreases less and less for each degree rise in temperature, the increments of streaming velocity "progressively increase between 10° and 30°". Such a progressive increase in the increment of streaming velocity might be due to the fact that the viscosity-temperature relation is of the type shown in Fig. 3, that is to say that there is a maximum in viscosity at around 15 or 20°. If this were the case, and if we assume further that the propelling force increases uniformly as the temperature is raised, it would obviously follow that at lower temperatures there would be a

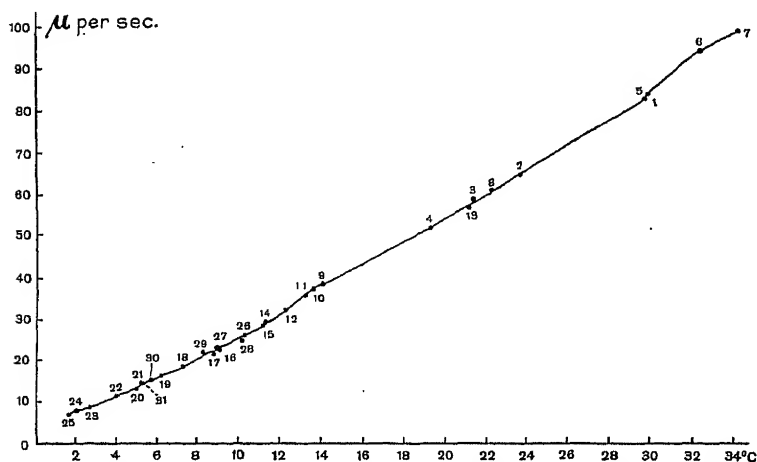


Fig. 4. Speed of protoplasmic streaming in a cell of *Nitella* at different temperatures (after LAMBERS '25)

smaller increment in streaming velocity than at higher temperatures.

In favor of the opinion that the action of temperature on the rate of streaming is primarily on the propelling force, are the oldest experiments on the effect of temperature on streaming, those of DUTROCHET '37. It may be interesting to quote DUTROCHET'S words:

"La circulation existe chez la *chara flexilis* à la température de la glace fondante, mais elle est lente. En échauffant lentement l'eau dans laquelle la plante est plongée, la circulation s'accélère graduellement; elle devient extrêmement rapide à + 18 degrés



cent. La chaleur de l'eau étant portée lentement à  $+ 27$  degrés, la circulation devient extrêmement lente; elle augmente ensuite peu à peu de vitesse sous l'influence continuée de cette même température de  $+ 27$  degrés cent.; et au bout de deux heures elle est devenue d'une grande rapidité."

According to these observations, the rate of streaming first decreases as the temperature is raised from  $18^{\circ}$  to  $27^{\circ}$ , and only after some time has elapsed does it become swift. We would expect that the effect of temperature on protoplasmic viscosity would be quick. If this were the case, DUTROCHET's results would indicate a decrease in protoplasmic viscosity as the temperature was raised from  $18^{\circ}$  to  $27^{\circ}$ . The increase in the rate of streaming that followed long exposure to the higher temperature would then be due to a response of the plant which involved an increase in the force which caused the streaming. But of course this is purely theoretical, and as a matter of fact DUTROCHET's results do not seem to be in accord with the results of later experimenters (see for example EWART '03, p. 63).

Why certain types of protoplasm should show a maximum in viscosity at  $15^{\circ}$  is an interesting problem from a physical standpoint. Various theoretical possibilities have been thought of, but it seems best to leave the question open.

It is a remarkable fact that the protoplasm of most animals and plants is killed by temperatures only a few degrees above that of the natural environment. Often enough organisms in nature are destroyed by a slight increase in the temperature of their surroundings. DAVENPORT '97 gives a list of the temperatures which produce death in various animals and plants. This list is far from complete, and some of the observations on which it is based are not reliable. It seems safe to say that animals generally die at temperatures between 30 and 45 degrees and that plants die at temperatures between 40 and 50 degrees. There are of course quite a few exceptions. Fishes in temperate latitudes usually die at about  $25^{\circ}$ . Some animals die at even lower temperatures. Thus BRAUER '77 found the death point of two Phyllo-pods to be  $18^{\circ}$  and  $19^{\circ}$  respectively. PFEFFER '04 (see vol. 2, p. 294) states that some plants die at temperatures as low as  $20^{\circ}$ . The alga *Hydrurus foetidus* dies at  $16^{\circ}$  (KLEBS '96).

There are also exceptional cases in which organisms can live at much higher temperatures. The optimum temperature

for thermophile bacteria is 60—70° C. and they are only killed by temperatures above 75° (see PFEFFER p. 87, and literature p. 96). There are also records of animal and plant life in hot springs, although these records should be regarded with caution, for the temperature varies greatly in different parts of a hot spring. An organism may have spent most of its life in a relatively cool part of a hot spring and may then have been displaced into a warmer part just before the observations were made (PFEFFER).

If they are able to exist in a dry state, various organisms are able to withstand temperatures far above those which are normally fatal. This has been known since the time of SPALLANZANI. Seeds, mosses, lichens, fungus spores, and bacteria when dried are often capable of continuing their existence after an exposure of an hour or hours to temperatures of 100° or even 120° (for the older literature see PFEFFER '04, p. 293). A few forms of animal life can also be dried and these too can resist high temperatures when water is absent. Thus RAHM '22 found that rotifers and tardigrades when dried could withstand temperatures up to 150°. Not only is it true that certain animals and plants in a dry state can suffer exposure to higher temperatures, but there is also evidence that those organisms and tissues which can not stand drying also become increasingly resistant to heat as their water content is diminished. This is a point which will be considered in greater detail later.

The cause of heat death in animals and plants has frequently interested zoologists and botanists. In our discussion we shall consider only the typical case in which the organism dies at a moderate temperature. The few rare instances in which plants or bacteria can exist at relatively very high temperatures would require special treatment and special study. Nor is the capacity of dried organisms to resist high temperatures a general attribute of protoplasm; the organisms which can be almost completely freed from water are hardly in a living state when they are thoroughly dry.

The most obvious explanation of heat death, and the one most often proposed, is that the proteins of the living cell become coagulated by heat in the same manner that egg albumin becomes coagulated when it is heated in a test tube. Actually there is very little experimental evidence in support of this assumption. BRODIE and RICHARDSON '99 isolated various proteins from muscle

and claimed that the temperatures at which these proteins coagulated also produced definite shortening in the intact muscle. Later, BRODIE and HALLIBURTON '04 obtained similar results for nerve. The work of these authors hardly affects our main problem. For the most part, they were interested in temperatures well above those which kill the tissues in question, and whether or not heat coagulation of proteins occurs in cells already killed by heat is a question of secondary importance. Moreover the above-mentioned work of BRODIE and his collaborators has been sharply criticized by MEIGS '09, who has cast grave doubt both as to the accuracy of their experimental results and the logic of their interpretation. Among botanists, LEPESCHKIN has consistently regarded the heat death of protoplasm as primarily due to a heat coagulation of the cell proteins (see LEPESCHKIN '10, '11, '12, '23).

The authors we have cited above, and many others as well, believe that the heat death of the cell is directly due to a coagulation of proteins. Of course no one doubts that when a cell is killed either by heat or by some other agency, its proteins are typically coagulated. The question is whether, in death at higher temperatures, the direct effect of the heat on the proteins is the primary factor. Ordinarily proteins are not coagulated by the temperatures which cause death in animals and plants. For this reason, various authors have suggested other possible explanations for the action of heat on protoplasm. WINTERSTEIN '05 proposed the view that heat caused death by asphyxiation. MAYER '17 suggested that the heat produced an accumulation of acid. Neither of these authors has offered very direct evidence in support of his theory.

It is certain that when protoplasm is subjected to temperatures just warm enough to cause death, it suffers a gelation or coagulation. SACHS '64 described a "Wärmestarre des Protoplasma", which he deduced from the cessation of protoplasmic streaming. DE VRIES '71 describes the formation of new granules in heat-treated plant tissues, and this is good evidence of coagulation. The same phenomenon is noted by LEPESCHKIN '23 for *Spirogyra* cells. With the centrifuge it is possible to show that a great increase in viscosity when cells are subjected to temperatures just high enough to cause death. This has already been indicated on the temperature-viscosity curve for the protoplasm

of the *Cumingia* egg (Fig. 3). HEILBRUNN '22 found a similar sharp increase in viscosity for the protoplasm of slime molds. The coagulation or increased viscosity produced by warmth precedes death. SACHS states that the "Wärmestarre" is reversible if the heat treatment is not too prolonged. LEPESCHKIN also finds a certain degree of reversibility. In his study of the heat coagulation of plasmolyzed *Spirogyra* cells ('23), he divides the process into four stages. The first stage is an invisible one which involves an increase in permeability, the second stage involves a visible change in the surface layers of the protoplasm. Many new granules appear, and the protoplasm no longer tends to present a regular smooth rounded surface. The third stage involves a complete coagulation of chloroplasts, and the fourth a complete coagulation of the protoplasm\*. If one interrupts the heat coagulation after the second stage, and returns the alga to room temperature, the granulation produced by the high temperature disappears gradually, and there is no lasting injury. Finally, in marine eggs it can be shown that the coagulation caused by heat occurs before death and is reversible. Thus in one experiment when *Arbacia* eggs were kept at a temperature of  $32.3^{\circ}$  for 20 minutes, the protoplasmic viscosity increased greatly, but it returned again to its normal value following removal of the eggs to sea-water at room temperature (see HEILBRUNN '24 b).

Thus in many types of living cells, heat produces first a coagulation and then death. In marine eggs, the coagulation produced by heat precedes any visible death changes, and is very probably the direct cause of death.

Is the heat coagulation of living cells simply a protein heat coagulation? Some facts speak in favor of this view. If one plots a curve for the time it takes for the protoplasm to coagulate at different temperatures, the general shape of the curve is very similar to that found for inanimate proteins. Fig. 5 shows the coagulation time at different temperatures of *Arbacia* and *Cumingia* egg protoplasm. The time of coagulation was determined by making centrifuge tests at frequent intervals. If one compares the curves in Fig. 5 with the curve obtained by BUGLIA\*\* for

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\* In his 1910 paper, LEPESCHKIN states that the chloroplasts coagulate before there is a coagulation in the surface layers of the protoplasm.

\*\* BUGLIA 1909, Kolloid-Zeitschr., vol. 5, p. 291.

the heat coagulation of serum albumin, the resemblance is obvious. Moreover both in protein heat coagulation and in the heat coa-

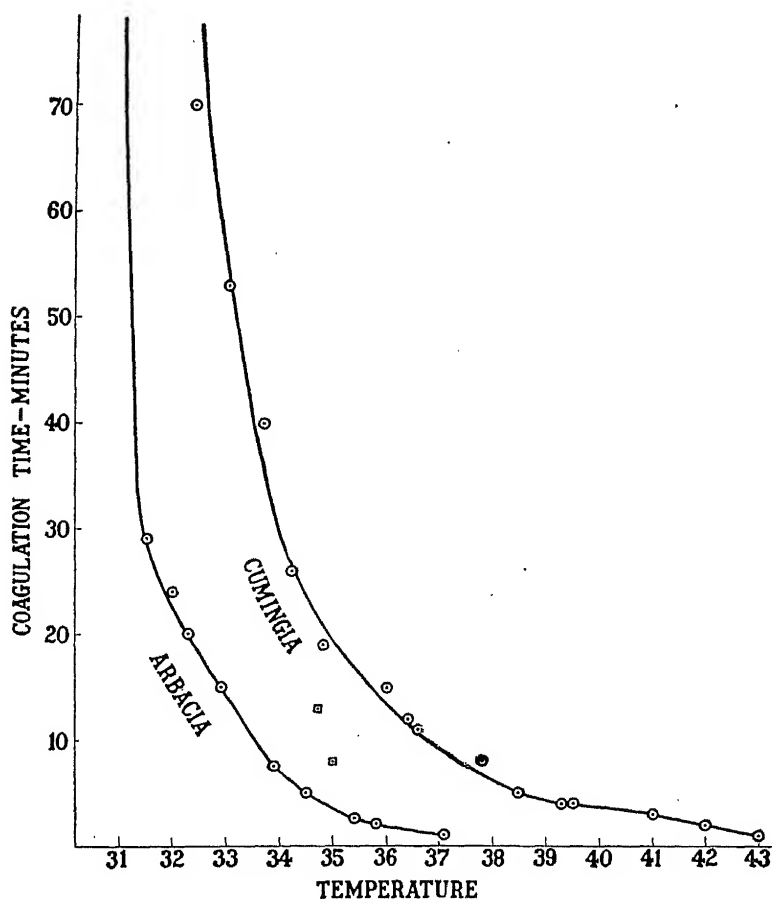


Fig 5. Heat coagulation time at different temperatures (HEILBRUNN '24b).

gulation of protoplasm, the temperature coefficient is of the same order of magnitude. CHICK and MARTIN\* found that the

\* CHICK and MARTIN 1910, Journ. of Physiol., vol. 40, p. 404.

temperature coefficient of the rate of coagulation per degree of temperature was 1.3 for hemoglobin and 1.91 for egg albumin. If one examines the curves in Fig. 5, it is easy to see that for each degree rise in temperature, the increase in rate of coagulation (decrease in time) is generally between these two numbers.

For the temperature coefficient of heat death in plants and animals, similar high values have been obtained. The temperature coefficient for 10 degrees, i. e. the  $Q_{10}$ , is 13.8 for hemoglobin and about 635 for egg albumin. COLLANDER '24 finds the  $Q_{10}$  for heat death in cells of the following plants: *Tradescantia discolor*, *Beta vulgaris*, *Brassica oleracea*, *Elodea densa*, *Draparnaldia glomerata*, *Pisum sativum*, to be 26, 71, 80, 31, 43, 118. COLLANDER also cites older authors who have found similar values for the  $Q_{10}$ , and he justly discards the somewhat lower values of LEPESCHKIN '13 as being due to faulty technique. In animal cells, high values for the  $Q_{10}$  of heat death have also been found. These range from 240 to 1000, (see KANITZ '15).

In spite of the similarity of the temperature coefficient, there are serious objections to the view that the heat coagulation of protoplasm is due to a direct effect on the proteins. The protoplasm of marine eggs is coagulated by temperatures of 30 to 35°, and as has already been shown, heat death may occur at even lower temperatures. That proteins undergo heat coagulation at temperatures as low as 30 or 35° is extremely doubtful. It is true that in the literature there are one or two descriptions of heat coagulation at these temperatures\*, but these observations were made on tissue extracts and in these extracts salts and other chemical compounds probably play a larger part in the coagulation than does the temperature. In pure preparations of proteins, heat coagulation occurs typically at temperatures above 50°.

In the second place, heat coagulation of protoplasm, as it occurs in marine eggs, differs from the heat coagulation of pure proteins in that it is reversible\*\*. For a time at least following

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\* VON FÜRTH 1895, Arch. f. exp. Path. u. Pharm., vol. 36, p. 231; POUL 1905, Hofmeisters Beiträge, vol. 7, p. 381.

\*\* Although it is true that under ordinary conditions the heat coagulation of proteins is irreversible, under certain special conditions, it may be reversible. Thus if the heat coagulated protein is boiled in the presence of certain electrolytes, it may go into solution again, see WIL-

the heat coagulation of the protoplasm in *Arbacia* and *Cumingia* eggs, the protoplasm becomes fluid again on return to normal temperatures. In these marine eggs, actual reversal of coagulation is certain. It seems probable that the return of protoplasmic streaming in plant cells heated to the point where movement ceases and then returned to normal temperature, is also an indication of reversible coagulation (see SACHS '64). So too we would interpret the reversal of heat rigor in frog muscle, as described by PICKFORD '51, GOTTSCHLICH '93. We conclude therefore that the heat coagulation of protoplasm is essentially different from the heat coagulation of proteins in that it is reversible, whereas protein coagulation is irreversible when the proteins are returned to room temperature.

The evidence that protein heat coagulation and the heat coagulation of protoplasm have similar temperature coefficients is no proof that the two processes are fundamentally similar. Many other physical processes also have very high temperature coefficients, for example melting or solution. There is thus no good argument in favor of a direct protein effect as a cause of heat death, and there is much evidence against this interpretation.

What other types of explanation are possible? In a later chapter (chap. 12), it will be shown that fat solvents generally cause a coagulation of protoplasm when present in sufficient concentration. Thus a dilute solution of ether always produces a decrease in viscosity, but with an increase in concentration of the ether, there is a sudden coagulation. What more natural than to assume that warmth has an effect similar to that of ether? High temperatures tend both to dissolve fats and to liquefy them. And as the temperature is raised, there is first a decrease in protoplasmic viscosity, and then a coagulation, just as is the case when the concentration of ether is increased.

We know moreover that fats may be profoundly modified by the very temperatures which cause heat death in protoplasm. Indeed, it is possible to show that the fats or lipoids of living cells are actually affected by the temperatures which cause death. When sea-urchin eggs are treated with temperatures just high enough to cause a slow death, even before coagulation takes

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HEIM 1927, Biochem. Zeitschr., vol. 180, p. 231. This does not affect our argument.

place the eggs when centrifuged show a marked alteration in the appearance of the fatty materials. Ordinarily these are compressed into a very small cap at one pole, but in heat treated eggs the fat particles occupy a much larger zone, and with longer heat treatment they disappear completely (HEILBRUNN '24b). Apparently the fat particles are dissolved at these lethal temperatures. According to HÖRSTADIUS '23, the lipoids in the protoplasm of the eggs of the annelid *Pomatoceros* undergo visible changes at a temperature of 40°.

If the heat coagulation of protoplasm is really initiated by a solution or liquefaction of lipoids, then fat solvents should hasten the process. This was actually found to be the case. In *Arbacia* eggs exposed to a temperature of 32°, HEILBRUNN '24b found that 1—1½ % ether decreased the time necessary for coagulation from 24 to 12 minutes. Such low concentrations of ether have a liquefying effect at room temperature, but at 32° they hasten the coagulation of the protoplasm. The action of acids in depressing the heat coagulation temperature of plant cells (KAHO '26b) may also involve fats.

Concerning the mechanism of the heat coagulation of protoplasm, we shall have more to say later. It will be shown in a subsequent chapter (chap. 14), that all agents which dissolve fats produce a characteristic coagulative reaction in the protoplasm. The solution of fats is only the first of a complicated series of reactions.

If we accept the view that the heat coagulation of protoplasm depends primarily on an alteration of the fats or lipoids of the cell, it is easy to understand why an increase in water content would favor heat death and a decrease in water concentration would retard it. The higher the percentage of water in the cell, the more readily would fats and lipoids tend to dissolve. For HANSTEEN-CRANNER '22 has shown that distilled water can cause a solution of protoplasmic lipoids in various plant tissues. In sea-urchin eggs it is possible to show from a study of centrifuged eggs that distilled water causes a solution of the lipoids of the cell.

Various authors have shown that with a decrease in the water content of the cell, there is an increased resistance to heat. SACHS '64 found that in air, plants were able to withstand higher temperatures than in water. However in SACHS' experiment it



is probable that the plants in the water actually reached a higher temperature than those in the air. A more dependable experiment is that of DE VRIES '71. He found that when various types of plant tissues were placed in 10 % sodium chloride solution, they were able to resist higher temperatures than control tissues in water. Similar results were obtained by VERNON '99 on frog muscle. He found that in hypotonic solutions heat rigor of the muscle occurred at lower, whereas in hypertonic solutions it occurred at higher temperatures than when the muscle was heated in isotonic solutions. The results of WEBER '26 also indicate that the heat resistance of stoma guard cells of various plants varies with the amount of water they contain. The higher the water content, the lower the heat resistance.

Considerable evidence has been presented for the view that heat death involves a solution or liquefaction of fats or lipoids. It might therefore be expected that animals and plants which suffer heat death at relatively low temperatures would have in their cells fats of relatively low melting point. In 1924, HEILBRUNN '24b attempted to show that this was the case. He pointed out that fishes have fats which are typically fluid at room temperature, whereas mammals and birds have fats which are commonly solid, and he suggested a correlation between this fact and the fact that fishes die at much lower temperatures than do the warm blooded animals. HEILBRUNN also quoted CZAPEK '13—'21 to show that the oils of tropical seeds have higher melting points than those of temperate climates. This is apparently a very general truth. Thus palm oil, cocoanut oil, cocoa butter are much more saturated and have higher melting points than linseed oil and other oils of plants which grow in temperate climates.

When HEILBRUNN's paper was published it was very difficult to obtain data regarding the melting points of the fats found in the tissues of lower animals, for in general these fats have no commercial importance. The whole subject has been put on a much surer basis by the recent paper of TERROINE, BONNET, KOPP and VÉCHOT '27. These authors published many determinations of the iodine number of the fats found in the organs of lower animals. Their results show clearly that in cold blooded animals the fats have a higher iodine number and are thus more fluid than those of warm blooded animals. But when cold blooded animals are accustomed to living at relatively high temperatures,

they have fats of higher melting point. TERROINE, BONNET, KOPP and VÉCHOT report experiments in which they grew a fungus, *Sterigmatocystis nigra* (= *Aspergillus niger*) and a bacillus, "bacille de la Fléole", at high and low temperatures. At the higher temperatures, the fats had a much lower iodine number, that is to say a higher melting point. TERROINE and his co-workers also refer to the experiments of HENRIQUES and HANSEN '01, in which it was shown that the cutaneous fat of pigs reared in the cold had a markedly lower melting point than the cutaneous fat of pigs reared at warmer temperatures. Moreover it is a well known fact that the fat in the interior of the body in mammals has a higher melting point than the subcutaneous fat, and this is to be associated with the higher temperature of the interior (see HENRIQUES and HANSEN '01).

LEATHES and RAPER (p. 119)\* believe that the fats are formed in living systems by a series of reactions in which the terminal points vary according to the temperature of the medium. At higher temperatures more saturated fats would be produced. These would have a higher melting point.

If in organisms the fats of the cells obey this rule, that is to say if fats of higher melting point are produced when animals and plants live at higher temperatures; and if, as we have reason to believe, heat death depends on the liquefaction of fats, we have an excellent interpretation of the acclimatization of living organisms to higher temperatures.

Organisms are killed by cold as well as by heat. With the exception of some bacteria which can resist temperatures close to the absolute zero, animals and plants generally, when in an active state, are killed by temperatures in the neighborhood of the freezing point of water. In the discussion of heat death it was pointed out that those forms of life which could exist in a dried state were far more resistant to heat when water was removed from them. So too seeds, mosses, rotifers, etc., when in a quiescent dry condition, can withstand much lower temperatures than when water is present. RAHM '21 found that dried tardigrades, nematodes, and rotifers were not killed by a temperature of  $-271.8^{\circ}\text{C}$ .

If we exclude the above-mentioned cases of unusual resistance to cold, there are in the main two types of phenomena which

\* LEATHES and RAPER, *The Fats*, London and New York 1925.

we shall have to consider. Some animals and plants die at temperatures above zero Centigrade. Others die only when their temperature falls below zero. Warm blooded animals, mammals for example, die when the body temperature falls to about 15° C. Whether death in these animals is due to a direct effect on the protoplasm of the cells is very doubtful. Tropical plants die at temperatures above zero. SACHS '64 cites various observations of this sort, but these are not very trustworthy. Often the temperature of a plant is below that of its environment, due to the cooling effect of evaporation from its surface. In the experiments of MOLISCH '97, any possibility of error of this sort is excluded. It is certain, therefore, that some plants do actually die at temperatures above zero Centigrade.

It was shown previously that in the *Cumingia* egg a marked increase in viscosity occurred at one degree above freezing. It is quite possible that such a coagulative effect would be an important factor in causing the death of cells at temperatures just above the freezing point of water. Mammalian muscle contracts on cooling to 5° C. (BOTTAZZI '25b). There is thus a cold rigor as well as a heat rigor of muscle. In the *Cumingia* egg, the coagulation due to cold is reversible, just as is the heat coagulation. But it is not at all improbable that after a lengthy exposure to 1° C., the protoplasm may be permanently injured. Concerning the reasons for a sudden increase in viscosity at temperatures just above the freezing point, we have at present no real information. A few scattered observations indicate that in this case too there may be a change in the physical properties of the lipoids of the cell. In *Cumingia* eggs centrifuged at 1° C., the lipoid particles present a very characteristic appearance, quite different from that found in similar cells at normal temperatures.

Most animals and plants are killed by the cold only when their temperature drops below the freezing point of water. Owing to various economic phases of the subject, there have been many more studies of the cold death of plants than of animals. In temperate or cold climates, the death of plants as a result of exposure to cold is an important agricultural problem.

The older literature on the effect of freezing temperatures on plants has been well summarized by PFEFFER '04. For later summaries, see CHANDLER '13, ROSA '21, NEWTON '22, DOYLE and CLINCH '26, AKERMAN '27. Generally speaking, death in plant

cells depends on the formation of ice crystals. At one and the same temperature, death occurs if crystals of ice are formed, whereas if no crystals appear, the cells live on (see PFEFFER, also KYLIN '17). The crystals may be formed either within the cells or outside of them. Concerning the cause of death, the leading theory is that of MÜLLER-THURGAU '80, '86. This author holds that as ice forms, the salt concentration of the rest of the protoplasm increases until the death point is reached. MÜLLER-THURGAU's theory is supported by MOLISCH '97 and by MAXIMOW '14. However, the latter author believes that in addition to the salt effect following ice formation, there is also a mechanical effect of the ice crystals on the colloids of the protoplasm. If the MÜLLER-THURGAU theory is correct, then cold death at freezing temperatures is really a special case of the toxic action of high concentrations of salt in the cell.

MATRUCHOT and MOLLIARD '02 and ZACHAROWA '26 have described the formation of numerous vacuoles in cells killed by cold. These vacuoles appear both in cytoplasm and nucleus. They may owe their origin to the formation of ice crystals, or perhaps they are the result of increased salt content. As will be shown later, the appearance of vacuoles is a logical result of the increase in the concentration of salts in a cell (see p. 249).

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## CHAPTER VIII

# THE ACTION OF VARIOUS PHYSICAL FACTORS OTHER THAN TEMPERATURE

Not only is protoplasm sensitive to changes in temperature, it is also sensitive to other physical factors of its environment. In the present chapter we shall endeavor to show how the protoplasmic colloid is affected by physical forces and agencies of one sort or another.

It is not always easy to separate physical factors from chemical. When a cell is treated chemically, such treatment may involve physical effects, a change in surface tension for example, or a difference in the osmotic pressure on the cell membrane. So, too, physical agencies may act primarily by the initiation of chemical reactions. It is impossible to draw any sharp line. For the purposes of classification, we will consider as a physical factor anything that starts as such.

We shall discuss the action of the following:

1. Mechanical factors.
2. Electric currents.
3. Light.
4. X-rays.
5. Beta and gamma rays of radium.

Biologists have studied the effects of pressure on protoplasm and the effects of wounding. Practically all of the work has been done by botanists. Since the time of DUTROCHET '37, it has been known that mechanical pressure exerted on a plant cell can cause the cessation of protoplasmic streaming. This observation has been repeated by various observers. A relatively recent paper which deals with the subject is that of LAUTERBACH '21. References to the older literature may be found there. LAUTERBACH observed that in younger cells and at higher temperatures less pressure was necessary to cause cessation of movement.

She found also that the response to pressure, that is to say the stoppage of movement, travelled from cell to cell. All these facts were known to EWART '03. When streaming protoplasm is subjected to pressure, it is probable, but of course not certain, that the cessation of movement is due to a coagulation or a sharp viscosity increase in the protoplasm.

In 1910, LEPESCHKIN described what he called a mechanical coagulation of the protoplasm of *Spirogyra*. Filaments of *Spirogyra* were placed under a cover slip and distorted several times by pressure from above. Following this treatment, according to LEPESCHKIN, the outer protoplasmic layer coagulated and the inner protoplasm broke up into a number of balls. Sometimes the cells burst and the balls passed out into the surrounding medium. After half an hour, all the balls were coagulated too. LEPESCHKIN's criterion of coagulation is wholly a morphological one, and depends in the main on the appearance of new granules.

Many authors describe the appearance of vacuoles following mechanical injury. Thus HEILBRONN '22 states that mechanical injury produces vacuolization in slime mold plasmodia. BUENNING '26b states that countless vacuoles appear in onion cells following mechanical coagulation. These had previously been described in fixed material by NĚMEC '01b. But this knowledge is very much older, as is evident from the following quotation from a paper written in 1876 (VELTEN '76b): "bei Druckwirkungen entstehen in den verschiedensten Zellen, wie dies bereit bekannt, Vacuolen; . . . . . War die Einwirkung nicht zu stark, so verschwinden sie nach einer Ruhepause wieder und das Protoplasma kann in seinen normalen Zustand zurückkehren". The formation of numerous vacuoles within the protoplasm of a cell would beyond any question cause a marked increase in the protoplasmic viscosity. If enough vacuoles were present, the protoplasm would of course be totally incapable of flow, it would be gelled or coagulated.

There are two different ways in which mechanical forces can act on protoplasm. Cells may be subjected to pressure insufficient to cause any rupture of the cell membrane, or they may be compressed so as to force out the interior protoplasm until it comes in contact with the outer medium. Cutting and tearing of cells also exposes the interior protoplasm to the surrounding medium. When a cell is cut, torn, or compressed so that its protoplasm comes into contact with the outer medium,

the naked protoplasm often reacts by forming a film about itself. It is not our purpose at this point to consider the process of film formation about emerging droplets of protoplasm. This is a subject that will be taken up in detail in a later chapter (see chap. 13).

There is an interesting possibility that the coagulative effects of pressure are in all cases due to an exposure of the cell interior to the outer medium. When a cell is violently compressed, we see the protoplasm emerge. But if a cell is subjected to a less violent pressure, it may very well happen that the cell membrane is broken here and there, and immediately mended. If these slight gaps were small, and if the mending process were rapid, the rupture of the membrane would certainly escape our notice. Evidence in favor of the view that all mechanical coagulation involves a rupture of the membrane of the cell is found in the fact that the appearance of vacuoles in cells under pressure as mentioned in the quotation from VELTEN, is also a phenomenon which occurs in cells which have been broken or torn (see chaps. 13 and 14). If this point of view is eventually shown to be correct, we may be in a better position to understand the remarkable effects which pressure has on living systems, effects quite unlike anything that is to be found in inanimate colloids. But, for the present, we have nothing more than an untested hypothesis.

Something has already been said regarding the effects on protoplasm of pressures not great enough to cause a visible emergence of the cell contents. We shall now discuss the results of mechanical injury, such as is produced by a cut or wound. When an animal or plant is cut, the effect is not limited to the cells actually injured or even to the cells at the border of the cut. Cells at a considerable distance from the cut surface may be affected. It seems not at all unlikely that the transmission of the injury effect from cell to cell is due to the diffusion of chemical substances formed in the injured cells. Thus FRANK '72 found that the morphological changes produced by injury appeared sooner in cells in the neighborhood of the midrib in the case of leaves of *Elodea*. This would indicate that the changes are due to a substance which diffuses from the cut or injured region. There is also other evidence which points in the same direction. Some of this will be brought forward in later chapters. But for the present we shall consider the mechanical coagulation by itself

and we shall not attempt to understand the mode of its transmission or to determine whether this transmission is more physical or chemical.

When plant tissues are injured, both morphological and physical changes occur in the neighboring cells. Although it seems quite probable that the morphological changes depend on the physical, no one has attempted to show a relation between the two. The morphological changes have been studied by cytologists, the physical changes by physiologists.

In plant tissues various form changes can be seen in the neighborhood of an injured or cut surface. FRANK '72 described movements in the chloroplasts of *Elodea* cells. TANGI '84 found that protoplasm and nuclei of onion cells moved in a direction toward any injured region. TANGI cut the scales of onions with a sharp knife. NESTLER '98 repeated TANGI's observations for many types of plants. It is interesting to note that NESTLER used not only a knife and a glass needle, but he also found similar changes following wounds produced by the heat of a burning glass. Movements of nuclei and cytoplasm toward cut or injured surfaces have also been described by NĚMEC '01b, by KARLING '26 and by various others. For references, see KARLING '26.

The physical changes that occur in protoplasm following an injury to cells in the neighborhood have also been studied by botanists. HEILBRONN '12 found that when sections were cut through bean seedlings (*Phaseolus* and *Vicia*), the viscosity of the protoplasm was abnormally high for 10—15 minutes after the sections were made. During this time, the starch grains in the starch sheath cells did not move at all under the influence of gravity, although they moved rapidly enough later. Similar observations have been made by various other workers who have used the gravity method of measuring protoplasmic viscosity (see for example F. and G. WEBER '17 also ZOLLIKOFER '18).

Recently BUENNING '26a and b has made an interesting quantitative study of the increase in viscosity which follows wounding. BUENNING studied the viscosity changes with the gravity, centrifuge, and plasmolysis-form methods. In his experiments he cut the plant tissue with a razor, but he is careful to show that there is no chemical effect of the metal, for he states that controls in which there was no possibility of chemical action gave the same results (compare also the experiments of Po-



RODKO '12c). In his first paper, BUENNING used seedlings of *Secale cereale* and *Raphanus sativus*, and sprouts of *Tradescantia viridis*. The seedlings were decapitated, and then viscosity determinations of the protoplasm of cells near the cut were made both with the gravity and centrifuge methods. In these viscosity determinations, the starch grains or granules were made to move in intact masses of tissue, and then sections were cut to determine the extent of the movement. As pointed out in a previous chapter (see p. 44), this method is preferable to the older method of cutting the sections first and then watching the fall of starch grains through the cells of the section.

BUENNING found that coagulation occurred in cells several layers distant from the cut surface. There is a sharp increase in viscosity. The time required for the attainment of maximum viscosity varies somewhat in the different species. In *Secale*, at least 6 minutes elapse after cutting before a maximum viscosity is reached. In *Tradescantia* the maximum viscosity appears to be reached within  $1\frac{1}{2}$  minutes.

The coagulation or increase in viscosity is reversible. Thus in *Tradescantia*, centrifuge tests showed that 6 to 7 minutes after cutting the viscosity had returned to normal. When, however, instead of a single cut across the stalk, thin sections were made, a longer time was necessary for recovery. Under these conditions, in *Tradescantia*, the viscosity only returns to normal within 10 minutes at the earliest. In a similar experiment in *Raphanus*, the return to normal required 30 minutes. BUENNING points out that a longer time is necessary for recovery than for the development of maximum viscosity.

The increase in viscosity following a cut through the stalk is quite considerable. In the cells of *Secale*, one minute after the cut, the viscosity rose to 4 times its original value. In *Tradescantia*, after half a minute, it rose to  $3\frac{1}{2}$  times its original value; and in *Raphanus*, after  $2\frac{1}{2}$  minutes, the viscosity was at least 5—6 times as great as before cutting.

In a later paper ('26b), BUENNING studied the effects of wounds on the protoplasm of onion cells. He used the cells of the scales of the onion, and as before, he stimulated by cutting with a razor. In the onion cells there are no starch grains or larger granules, and neither the gravity nor the centrifuge method could be used. BUENNING therefore used the plasmolysis-form method. The time

required for coagulation is not more than 30 seconds. The increase in viscosity is followed by the clouding over of the protoplasm and the appearance of new granules both in the cytoplasm and in the nucleus. There are also many small vacuoles formed during the coagulation process.

Electric currents have a marked effect on various types of living material. In muscle and nerve physiology, the electric current is the most common means of stimulation. Lower forms of animal life and various plant tissues also show a characteristic response to the passage of electricity. It is to be expected, therefore, that the protoplasmic colloid should undergo changes following exposure to electric currents. There is an abundance of evidence to prove that this is the case.

In 1862 BRÜCKE showed that electric currents produced a cessation of Brownian movement in the salivary corpuscles or leucocytes of human saliva.

KÜHNÉ '64 found that the passage of an electric current from an induction coil caused a cessation of Brownian movement in the protoplasm of the stamen hair cells of *Tradescantia*. CHIFFLOT and GAUTIER '05 also found that the passage of an electric current stopped Brownian movement in the cells of the alga *Cosmarium*. BAYLISS '20 studied the effect of currents from an induction coil on Brownian movement in the protoplasm of ameba. The amebae were placed on a slide in a drop of water, and the electric current was led to the drop through strips of platinum foil. With this technique, there is of course the possibility that various chemical substances formed at the electrodes may have an effect on the protoplasm. But BAYLISS' observations were usually made very rapidly, so that this was probably not a factor. In studying the Brownian movement, BAYLISS used a ZEISS paraboloid condenser, and he focussed his attention on the ectoplasm of the pseudopodia. Here one can observe a very vigorous movement of granules invisible with brightfield. On the passage of an electric current, this movement "ceases almost instantly, as if the protoplasm had been frozen". Then when the current is stopped, the Brownian movement begins again. In order to obtain these results, it is necessary to use currents that are neither too weak nor too strong. The latter produce an irreversible coagulation of the protoplasm.

From these studies on Brownian movement, it is apparent that electric currents can produce a reversible gelation or coagulation of the protoplasm\*. A much more exact study of the effect of the electric current on the colloidal properties of protoplasm was made by BERSA and WEBER '22. These workers were careful to exclude any possible error due to the formation of chemical decomposition products at the electrodes. The current passed from carbon electrodes through troughs containing tap water, and then to the tissue by way of long strips of moist filter paper. Water and filter paper were changed from time to time, so that there was no possibility of the diffusion of chemicals from the electrodes to the cells. The material used was the stalk (epicotyl) of the bean plant *Phaseolus multiflorus*, and the current was passed through pieces of the stalk about 1—2 cm. long. The viscosity of the protoplasm was determined by the centrifuge method. The results of the experiments are very clearly summarized by BERSA and WEBER, and the account that follows is practically a translation of their words.

In order to produce a viscosity increase in the protoplasm, an exposure of only  $\frac{1}{4}$  to  $\frac{1}{2}$  minute is necessary, if the current is relatively strong (5—10 milliamperes). With weaker currents (0.15—5 milliamperes), an exposure of 1 to 4 minutes is necessary to produce the same effect. The increase in viscosity produced by these currents is considerable, for it is at least a three fold increase. The effect is reversible, and after a recovery period of 20 to 40 minutes, the viscosity becomes as low or nearly as low as it was before exposure to the electric current. The results of BERSA and WEBER find confirmation in some experiments of ZEIDLER '25.

It is obvious that the results obtained from observation of Brownian movement, and those obtained by the centrifuge method are consistent. Moreover, as we shall now proceed to show, these results are in complete harmony with the older knowledge concerning the effect of electric currents on the rate of protoplasmic streaming. This knowledge goes back to the year 1837. In that year BECQUEREL found that the passage of

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\* VELTEN '76c describes a case in which the passage of an electric current through *Elodea* cells caused an initiation of Brownian movement. This was apparently due to the fact that the granules of the protoplasm were dislodged into the vacuole where they had more room to move.

an electric current through cells of *Chara* produced almost immediately either a retardation or a complete stoppage of the protoplasmic streaming. Following BECQUEREL, the action of electric currents on protoplasmic streaming in plant cells has been studied by JÜRGENSEN '61, BRÜCKE '62, HEIDENHAIN '63, KÜHNE '64, VELTEN '76c, HÖRMANN '98, EWART '03 and KÔKETSU '23.

These authors worked with a wide variety of plant material, and in general they found that electric currents caused the rotation of the protoplasm to become slower or to stop completely. There are one or two exceptions which might be worth considering. Occasionally, according to VELTEN '76c, there is a slight acceleration of the rate of streaming in leaf cells of *Vallisneria spiralis*. This VELTEN attributes to a heat effect as a result of the passage of the current through material of high electrical resistance. There were indeed indications that the cells actually did become warmer. KÔKETSU '23 also describes one case in which there was an acceleration of the rate of streaming in a part of a *Chara* cell. He favors a different explanation from that of VELTEN.

But in the great majority of experiments, and they are many, the passage of an electric current has a very marked retarding effect on the speed of streaming. An experiment of KÔKETSU's may be taken as illustrative. In this experiment he stimulated a *Chara* cell seven times with the current from an induction coil. The current was of various strengths, being less of course as the distance between the primary and secondary coils was increased. Following each stimulation, KÔKETSU measured the speed of protoplasmic streaming by determining the time it took for the protoplasmic granules to travel a specified distance. The time was recorded in beats of a metronome, each beat being one-two hundredth of a minute. In the following table, the numbers at the head of the columns refer to the successive stimulations, the distances in centimeters being the distances between the primary and secondary coils in each case. The first column gives the time at which speed determinations were made. In the other columns, are records of inverse speeds. These figures show the times, in metronome beats, required for the protoplasm to move a given distance. These times would of course vary directly with the viscosity. Reading down any one column, one finds a record of a single experiment. When a complete stoppage of streaming occurred, this is indicated by S.

Speed of protoplasmic streaming in *Chara* after  
electrical stimulation (KÔKETSU '23)

Time of observation	No. 1 8.0 cm.	No. 2 7.5 cm.	No. 3 7.0 cm.	No. 4 6.0 cm.	No. 5 5.0 cm.	No. 6 4.0 cm.	No. 7 3.5 cm.
Before stimulation. .	8	8	8	9	8	8	8
Directly after stimulation . . . . .	8	8	8	9	S	S	S
0.5 min. after. . . . .	8	9	11	13	100	S	S
1.0 " " " " " " . . . . .	8	12	20	30	34	140	S
1.5 " " " " " " . . . . .	8	11	14	45	24	120	S
2.0 " " " " " " . . . . .	8	8	12	29	20	60	S
2.5 " " " " " " . . . . .	—	8	10	18	18	55	130
3.0 " " " " " " . . . . .	—	8	9	16	16	27	90
3.5 " " " " " " . . . . .	—	—	8	14	14	25	60
4.0 " " " " " " . . . . .	—	—	8	13	12	23	40
4.5 " " " " " " . . . . .	—	—	—	12	11	20	—
5.0 " " " " " " . . . . .	—	—	—	10	11	16	30
5.5 " " " " " " . . . . .	—	—	—	10	10	14	—
6.0 " " " " " " . . . . .	—	—	—	9	10	13	24
6.5 " " " " " " . . . . .	—	—	—	—	9	12	—
7.0 " " " " " " . . . . .	—	—	—	—	8	12	20
7.5 " " " " " " . . . . .	—	—	—	—	8	12	—
8.0 " " " " " " . . . . .	—	—	—	—	8	11	20
8.5 " " " " " " . . . . .	—	—	—	—	—	11	—
9.0 " " " " " " . . . . .	—	—	—	—	—	10	18
9.5 " " " " " " . . . . .	—	—	—	—	—	9	—
10.0 " " " " " " . . . . .	—	—	—	—	—	8	18
15.0 " " " " " " . . . . .	—	—	—	—	—	—	14
20.0 " " " " " " . . . . .	—	—	—	—	—	—	8

If one compares the observations of KÔKETSU with the viscosity measurements of BERSA and WEBER, one is struck with the similarity. Apparently the action of electricity on the rate of protoplasmic streaming in plant cells is due primarily to an effect on the viscosity of the protoplasm, rather than to an effect on the motive force of the streaming.

In animal cells too, the flowing movements of the protoplasm may be stopped by the passage of electric currents (see for example KÜHNÉ '64, ENGELMANN '69), but here the phenomenon is complicated by changes in the form of the cells. Moreover for animal cells no quantitative studies have been made which are at all

comparable in exactness with those which have been made on plant material.

For both plant and animal cells it seems certain that the passage of an electric current very quickly produces a reversible gelation or coagulation of the protoplasm. This is a fact of great importance, and it deserves further study. It seems probable that all types of irritable protoplasm may be electrically coagulated. Concerning the possible mechanism of such a process we know little or nothing. The fact that protoplasm is so sensitive to electric currents seems to indicate that the electric charges of the living substance are very important from a colloid chemical standpoint. If protoplasm were simply a colloidal solution of proteins, it would be hard to understand why electric currents exerted so powerful an influence. If we consider protoplasm as primarily a suspension, and we have abundant reason for so doing (see chaps. 2 and 5), we are perhaps in better position to understand the peculiar coagulative effect of electric currents, for the stability of a suspension depends to a greater extent on its charge than does the stability of a lyophillic colloid such as a protein solution. When an electric current is sent through a suspension or a suspenoid, the colloidal particles wander to one electrode or the other, and on reaching the electrode they give up their charge and are coagulated. But the contents of a cell do not wander to the electrode, unless we consider the bounding membrane of the cell as an electrode. This in a sense it is. If we pass an electric current through a suspension so that its particles move toward a membrane, as for example in electrodialysis, the colloidal particles when they reach the membrane lose their charge and become precipitated. Perhaps a similar process occurs in protoplasm; but this seems doubtful, for the electrical coagulation may be practically instantaneous even in large cells.

In a later chapter we shall have occasion to consider the electric migration or cataphoresis of protoplasmic granules through the cell (see chap. 10).

The mechanism of electrical coagulation of protoplasm may very well be related to the vacuole formation which frequently accompanies or follows the passage of an electric current through the cell. This is a complicated subject, and one that can not be adequately discussed at this point. It will be considered later, (see chap. 14).

In recent years there has been great interest in the action of light on plants and animals. But up to the present, there has been very little study of the effect of the light on the colloidal properties of the protoplasm. Many authors have shown that light rays, and especially ultraviolet rays, cause the death of bacteria exposed to them (for references see PINCUSSEN '21, CLARK '22). This death no doubt sooner or later involves a coagulation of the protoplasm, but one has no means of knowing whether or not this coagulation is a direct result of the radiation.

PRINGSHEIM '81 exposed plant cells to concentrated sunlight, taking care to avoid heat effects. He found that the increased intensity of light caused a decrease in the rate of protoplasmic streaming in *Nitella*. In *Spirogyra* cells, the concentrated sunlight tended to stop the "Körnchenbewegung" in the wall layer of the protoplasm. This "Körnchenbewegung" probably depends at least partly on the Brownian movement of the granules.

Many authors have studied the action of light on protoplasmic streaming. In most instances their results seem to depend more on an action of the light on the propelling force of the streaming than on the viscosity. Light rays have frequently been found to have an influence on the initiation of protoplasmic streaming. For references to literature, see BEIKIRCH '25. BEIKIRCH himself found that the stimulating effect of light on streaming consisted in an increase in the number of cells which showed streaming rather than in any increase in the maximal speed with which the protoplasm moved.

Ultraviolet rays seem to have a specific effect in slowing or stopping the streaming movement of protoplasm, and this effect may very well be on the viscosity of the protoplasm. HERTEL '04 found that when *Elodea* cells were exposed to ultraviolet rays with a wave length of  $280\ \mu\mu$ , the rotation of the protoplasm first became slower and then stopped completely. Similar results were obtained by SCHULZE '10 for the stamen hair cells of *Tradescantia*, the root hairs of *Hydrocharis*, and the leaf cells of *Valisneria*. He also used rays with a wave length of  $280\ \mu\mu$ . VOUK '12 studied the effect of ultraviolet rays from a mercury vapor lamp on the streaming of slime mold protoplasm. In most of his experiments he exposed the plasmodia for from 1 to 5 minutes and found a cessation of movement which was usually reversible. However, the longer exposures caused death. In one experiment, in which

the exposure was only 30 seconds, the speed of the streaming was increased. Such an increase in streaming was also observed when the rays passed through glass, so that the acceleration may have been due to an action of the visible rays of the spectrum.

In his experiments on the stamen hair cells of *Tradescantia*, SCHULZE noted that the rays of  $280\ \mu\mu$  wave length caused the appearance of many small vacuoles. These could be noticed even after an exposure of only 30 seconds.

That ultraviolet rays cause coagulation of protoplasm seems certain. CERNOVODEANU and HENRI '10 exposed paramecia and white blood cells to ultraviolet rays, and studied the appearance of the protoplasm under darkfield illumination. They found that the protoplasm became more granular and appeared brighter, ("plus brillant"). This is evidence of coagulation.

RUPPERT '24 treated *Ascaris* eggs with ultraviolet rays ( $280\ \mu\mu$ ) at various stages of mitosis, and then centrifuged them immediately. Before treatment the eggs were kept in an incubator at a temperature of  $37^{\circ}\text{C}$ . The centrifugal treatment was always the same. The eggs were centrifuged for 30 minutes at a speed of 3200 revolutions per minute. The radius of turn was about 10.5 cm. This is rather a severe centrifugal treatment (compare p. 103), and it is not surprising that the eggs always showed a complete stratification. But there was a very noticeable difference between the eggs which had been exposed to ultraviolet rays and the control eggs. In the exposed eggs, there was often a much slower return of the granules after the centrifuging process was discontinued. Sometimes the granules did not return at all. A similar observation had previously been made by SCHLEIF '23. Both SCHLEIF and RUPPERT conclude that there is a marked increase in viscosity in the protoplasm of the eggs exposed to ultraviolet rays, and this conclusion certainly appears to be justified. In one experiment, RUPPERT instead of centrifuging the eggs immediately after they had been exposed to ultraviolet rays, kept them over night at room temperature and then centrifuged them. In this experiment the eggs treated 5 and 10 minutes showed no movement of granules on being centrifuged, so that the viscosity of their protoplasm had evidently been greatly increased.

GIBBS '26 studied the effect of ultraviolet rays from a mercury vapor arc on the protoplasm of *Spirogyra* cells. He found that



radiation between  $3126\text{\AA}$  and  $2378\text{\AA}$  (i. e.  $312.6\text{--}237.8\text{ }\mu\mu$ ) caused first a liquefaction and then on longer exposure a coagulation of the protoplasm. Viscosity measurements were made with the centrifuge method. The following table, taken from GIBBS' paper, shows the decrease in protoplasmic viscosity produced by relatively short exposures to the mercury vapor arc.

Effect of ultraviolet light on *Spirogyra* protoplasm (GIBBS '26)

Time of centrifuging in seconds	Cells exposed to unscreened arc for 30 minutes	Cells exposed to arc screened with "Noviol 0" glass	Cells not exposed
45	Slight displacement	Normal	Normal
60	Marked displacement in some cells	Normal	Practically normal
75	Marked displacement	Very slight displacement	Very slight displacement

ADDOMS '27 treated wheat seedlings with ultraviolet radiation from a mercury vapor lamp. The seedlings were exposed for 10—15 minutes, and the root hairs were then examined in dark-field (cardoid condenser). From the morphological appearance of the protoplasm, ADDOMS concludes that coagulation has occurred after 10 minutes. The seedlings are not injured by the treatment. Root hairs covered with glass show no coagulation.

Very soon after the discovery of X-rays and of radium, experiments were made to find out what effect or effects the new radiations might have on living organisms. And as soon as it became apparent that both X-rays and radium might have clinical value, these experiments became much more frequent. So that already there is a vast literature in this field, a literature which is scattered through biological and medical journals and journals devoted especially to the study of the effects of radiation.

Most of the work that has been done deals with gross effects on whole animals or plants or human beings. Plants grow more rapidly or more slowly, animals are killed, or men are cured. Biologists have not infrequently described effects on cell division or on the course of inheritance. Truly physical studies on the action of X-rays or radium on the protoplasm have been relatively few.

Some recent authors incline to the view that the primary effect of radium radiations and of X-rays is on the medium

surrounding the cells. For literature on this subject, see SARTORY, SARTORY and MEYER '27.

It is very commonly believed that the nucleus of a cell is much more sensitive to radium and X-rays than is the cytoplasm, and that the nuclear material is particularly sensitive during the metaphase when the chromosomes are not surrounded by a nuclear membrane. Numerous papers have been written in support of this thesis, for references to some of them, see SCHINZ '24, SEIDE '25. REISS '25 states that the nucleus of the sea-urchin egg is 18 times as sensitive to radium as is the cytoplasm, and 3.8 times as sensitive to X-rays.

There have been relatively few studies of the effect of radium and X-rays on protoplasmic streaming. The results as far as they go indicate that the action of X-rays and of beta and gamma rays of radium are similar to those of ultraviolet rays.

WEBER '23 found no effect after treating two types of plant protoplasm with X-rays. He radiated *Spirogyra* cells with 170 HOLZKNECHT units, and then centrifuged five minutes later. There was no observable difference between the radiated material and the controls. After 20 hours, an increase in viscosity did occur, but this WEBER interprets as being due to a secondary effect. WEBER also radiated seedlings of *Phaseolus multiflorus* with 24 HOLZKNECHT units, but again he was unable to detect any change in the viscosity of the protoplasm. Apparently some types of protoplasm are very resistant to X-rays. This is certainly true for paramecium. In this form, prolonged exposure to strengths of X-rays that would have a very serious effect on the life of higher types of animals, have no marked effect either on the protoplasmic viscosity or on the activities of the animal (unpublished experiments).

However, WILLIAMS '23 was able to show very definite effects of X-ray treatment on the protoplasm of the stalk cells of *Saxifraga umbrosa*. Short exposures caused an increase in the rate of protoplasmic streaming, and also an increase in the Brownian movement of the small particles of the protoplasm. Longer exposures caused a diminution of the rate of streaming, and finally complete stoppage. Similar results are also recorded in an early paper of LOPRIORE '98. But this worker studied only the rate of streaming and not the Brownian movement. He found that a half hour treatment with X-rays caused an increase

in the rate of streaming in the protoplasm of the leaves of *Valisneria spiralis*. Treatment for an hour caused the protoplasm to become yellow, granular, and coarsely vacuolated. SECKT '02 also found that short exposure to X-rays increased the rate of protoplasmic streaming in various plant cells, and that longer exposures slowed it. From all these results, it seems probable that X-rays first produce liquefaction and then coagulation of the protoplasm.

Similar effects have been found to follow radium treatment. Thus FORBES and THACHER '25 found a decrease in viscosity of the protoplasm of the eggs of *Nereis* (an annelid), following treatment with radium. In their own words, "*Nereis* eggs radiated with  $\beta$ -rays, fertilized and then centrifuged from 7 to 65 minutes after fertilization, show slightly sharper separation of resulting zones than is found in unirradiated controls. The difference is most easily explained as due to decreased viscosity of the protoplasm in consequence of radiation."

WILLIAMS '25 studied the effect of beta and gamma rays of radium on the protoplasm of *Saxifraga umbrosa*. Short exposure caused an increase in the amplitude of the Brownian movement of the small particles of the protoplasm, together with an increase in the rate of protoplasmic streaming. Evidently a decrease in viscosity is produced. Longer exposure to radium caused coagulation, and vacuolization of the protoplasm. Comparable results were also obtained by ZUELZER and PHILIPP '25 on ameba. These authors found that short exposures to radium caused an increase in the speed of ameboid movement, longer exposures resulted in a vacuolization of the protoplasm.

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## CHAPTER IX

# THE ACTION OF VARIOUS SALTS

Protoplasm contains salts and these salts play an important rôle in the life of the cell. So much is common knowledge. There can be no doubt but that certain salts or ions are essential for life. These salts or ions must be present within definite limits of concentration, and there must moreover be a balance between the concentration of one ion and another.

Because of the obvious importance of the protoplasmic salts, and because of the relative simplicity of these constituents, physiologists have very frequently studied the effect of the more common salts on various aspects of the life of the cell.

In studying the action of specific salts or ions, it is of course essential to exclude other factors from participation in the experiment. In the first place, a salt must not be used in such concentration that it exerts an osmotic effect. The solution used for the experiment must neither be hypertonic nor hypotonic, it must neither extract water from the protoplasm or give up water to it. Frequently physiologists have drawn conclusions regarding the particular action of some one salt from experiments in which cells or tissues were immersed in solutions which exerted a decided osmotic action. It is not always a simple matter to decide just which strength of solution to use. Most workers assume that solutions which have the same freezing point are equal in their osmotic effects. But this could only be true for an ideal semipermeable membrane, a membrane which is permeable for the solvent, in this discussion water, and impermeable for all dissolved substances. No such membrane is known. Certainly the osmotic membranes of cells show differences in permeability toward various types of salts and other dissolved substances. Hence a theoretically isosmotic solution may in actual practice extract water from the cell, or it may have the opposite effect. Indeed it seems certain that other factors are involved in addition

to a differential permeability, so that it is quite out of the question to know from available physical data just what strength of solution will be in osmotic equilibrium with a given type of cell.

How then can one know what strength of solution to use? For some cells it is possible to test the osmotic effect of various concentrations of any given substance merely by determining the volume of the cell in different concentrations. That concentration which causes neither loss nor gain of water is isosmotic. When cells have irregular shapes, it is not always easy to determine their volume. Such cells are not to be recommended for experiments with salts, for it is impossible to exclude osmotic factors. Most plant cells are surrounded by a stiff wall which prevents any marked increase in volume. The only action of a hypotonic solution on these cells is to cause an increase in the pressure exerted on the protoplasm by the cell wall. With the ordinary type of plant tissue, the osmotic factor is probably unimportant as long as hypotonic solutions are used. Perhaps also hypotonic solutions may safely be used in experiments with fresh water protozoa.

A second factor which must not be disregarded is the hydrogen ion concentration. The older experiments on the action of salts took no cognizance of this factor. Colloid chemists have repeatedly emphasized the importance of the hydrogen ion concentration in determining the behavior of various colloidal solutions. Nor have biologists been slow to realize that the concentration of hydrogen ions is a variable of great importance to life phenomena. Hence if we are to study the action of salts, we must be certain that our solutions either have the same pH as the normal medium, or that they have a pH which can be shown to have no marked effect on the protoplasm. In studying an inanimate colloid, it is only necessary to know the pH of the solution, that is to say of the dispersion medium. But when we immerse living cells in various solutions, we are interested not so much in the pH of the medium surrounding the cells as in the pH of the protoplasm itself. In an inanimate colloid the pH is what we measure it to be. But when cells are placed in different solutions of the same pH, the hydrogen ion concentration of the cell interior may be very different. Consider for example two solutions in which the pH is determined primarily by carbonic acid. Let us suppose that both solutions have the same pH, but that one of the solutions

contains carbonates, whereas the other does not. As a result of the law of mass action, it can readily be shown that the solution containing carbonates has a much higher concentration of carbonic acid than the solution without carbonates, in spite of the fact that it has the same pH. Because of such a higher concentration of carbonic acid, far more acid diffuses into the cell. The two solutions are therefore not comparable, even though at first sight they might appear to be. In experiments with cells it is not always sufficient to keep the pH constant. Some attention should also be paid to the source of the pH.

In studying the action of salts it is necessary to use isosmotic solutions and solutions whose pH is not likely to have an effect on the cell protoplasm. But even when we use such solutions there is another difficulty which it is almost impossible to circumvent. In most instances we do not know how rapidly a salt enters a cell and there is even some question as to whether salts enter at all. The weight of the permeability evidence certainly favors the idea that salts do diffuse into cells, but there is every reason to suppose that some salts enter more rapidly than others. If we compare the action of two salts, any differences that we observe may be due to a difference in the permeability. In general this does not seem to be possible in those cases in which the two salts produce opposite effects within the cell. But in those cases in which the difference in the action of the two salts is a difference of degree, we can not very well exclude the permeability factor.

From what has gone before, it is obvious that the greatest caution must be exercised in interpreting the results of experiments with salts. The conditions of experimentation should be such as to preclude osmotic effects or effects due to a difference in hydrogen ion concentration. And we must always recognize that the speed with which various salts enter the cells is a factor to be considered.

Many types of protoplasm are very sensitive to salts of heavy metals. This fact was first emphasized by NÆGELI, who found that *Spirogyra* cells were killed by copper and mercury salts in extreme dilution. Since NÆGELI's time, it has been customary to speak of this action of substances in extremely small concentration as an "oligodynamic action". In recent years there seems to have been a revival of interest in the phenomenon of oligodynamic action. References both to new and

older literature may be found in the papers of SEYBOLD '27 and LUDWIG '27. From data collected by LUDWIG, it appears that paramecium is killed by copper sulphate and mercuric chloride solutions when the concentration of these solutions is greater than  $10^{-6}$  molar. *Spirogyra* is even more sensitive and is killed by concentrations of  $10^{-6}$  molar.

In view of the fact that salts of heavy metals coagulate proteins in vitro, it seems not improbable that they exert the same effect in vivo. By actual viscosity tests it can readily be shown that low concentrations of  $\text{HgCl}_2$  produce a coagulation, or at least a great increase in viscosity, in various types of protoplasm. *Arbacia* eggs treated with m/10,000  $\text{HgCl}_2$  in sea-water very soon become altered so that the granules of the cells can no longer be moved by centrifugal force. The same experiment can also be performed on protozoan cells. When the flagellate *Euglena* is centrifuged, it loses its spindle-shaped contour and becomes spherical, the granular inclusions massing at one end. But when a small amount of mercuric chloride solution is added to the culture medium in which the *Euglena* lives, no shifting of granules occurs when the protozoa are centrifuged. In both *Euglena* and *Arbacia* cells, a great increase in viscosity follows treatment with mercuric chloride. The protoplasm ceases to behave as a fluid and is a gel or coagulum. The coagulative action of mercuric chloride, at any rate in higher concentrations, apparently involves a precipitation of proteins from the hyaline ground substance, that is to say the intergranular material, of the protoplasm. Not only is this a logical assumption, but there is also actual experimental evidence in its favor. When *Arbacia* eggs are first centrifuged, and are then treated with dilute solutions of mercuric chloride in sea-water, new granules can be seen to appear in the hyaline region previously free from granules.

In very dilute solutions of mercuric chloride in sea-water, there can of course be no question of an osmotic effect. Moreover these solutions have a pH very close to that of sea-water. Other solutions of metallic salts are frequently acid. Thus copper sulphate and copper chloride hydrolyze in aqueous solution, so that there is always some sulphuric or hydrochloric acid present. But it is easy to show that the copper ion has a specific effect quite apart from any acidity that may be present in solutions of its salts. Dilutions of copper chloride which have little or no acidity

may have a very pronounced action on the protoplasm. Thus in one of a series of unpublished experiments, the following five solutions of copper chloride in sea-water were prepared: m/1,000, m/5,000, m/10,000, m/50,000, m/100,000. Even the m/5,000 solution was dilute enough so that there was practically no effect of the hydrogen ion concentration (the pH of this solution was 7.8). All of the solutions produced a coagulative effect on the protoplasm of sea-urchin eggs, as was clearly shown by centrifuge tests. This coagulation did not occur very rapidly. Thus a test of the eggs in the m/5,000 solution showed no coagulation and no increase in viscosity after a 20 minute exposure, whereas a test after 54 minutes did show coagulation.

For some protoplasm the copper ion apparently does not exert so pronounced a coagulative effect. KLEMM '95 placed leaves of various aquatic plants in a 10 % copper sulphate solution. The streaming of the protoplasm was not interrupted, even though enough of the copper salt diffused into the cells to distinctly color the cell sap (compare also PRINGSHEIM '24).

The action of the ions of heavy metals on protoplasm, as far as it is known, seems easy to understand. Apparently metallic ions like those of mercury and copper cause a precipitation of the proteins of the cell, although perhaps this is not so certain in cases in which the metals are only present in very great dilution.

The behavior of protoplasm toward salts of the alkaline and alkaline earth metals presents a more difficult problem. These ions have no very great effect on proteins, but they have a profound influence on practically all types of living substance. Over and over again, workers with both plant and animal material have found that solutions of sodium chloride alone or potassium chloride alone, are toxic; but that the addition of a small amount of calcium or magnesium chloride is sufficient to offset the toxicity of the sodium or potassium ion. Often too, though not always, calcium and magnesium salts when present alone are toxic, and the addition of sodium or potassium ions is necessary to save the life of the tissue or organism. It is not our intention here to consider the vast literature on the subject of these ion antagonisms. Some of this literature has been summarized in a recent book of ZONDEK '27, and many details will be found in the last edition of HÖBER ('26). The review of RUBINSTEIN '28 may also be consulted. Not only have physiologists studied the effects



of sodium, calcium, and other ions on the life and death of various tissues, they have also made numerous experiments to show that in a wide variety of biological processes the physiological behavior is strongly influenced by the presence of one or the other of the ions in question.

Within the limits of a single chapter it would be impossible to discuss adequately the large and often contradictory literature on the antagonistic action of the salts of the alkaline and alkaline earth metals. We shall confine our attention almost exclusively to the studies that deal with the effects of the ions of these metals on the colloidal state of the protoplasm. Here too our task will not be simple. Not only because of the intrinsic difficulties of the subject itself, but because also the subject has now and again been attacked by biologists with no training in the elements of physical chemistry. Thus not infrequently one finds comparisons between the effects of equimolar solutions of calcium and sodium chloride, in spite of the fact that the calcium chloride dissociates into three ions instead of two, and therefore has a theoretical osmotic pressure roughly one and a half times as great as the sodium chloride solution when both are present in equimolar solution. Because of the unsatisfactory state of the literature, we shall not attempt any chronological survey, but instead we shall present a thesis and see how it fits the facts.

In view of the pronounced action of the alkaline and alkaline earth metals on all types of living substance, it seems logical to assume that the ions of these metals have definite effects on the colloidal properties of the protoplasmic colloid. That this should be true is readily understandable if we remember that protoplasm is a suspension (see p. 21). Colloidal solutions of proteins are on the whole very insensitive to the ions of sodium, calcium, etc., whereas on the other hand lyophobic colloids and suspensions are very sensitive to these ions. The stability of a suspension depends on its electric charge, and this charge is primarily due to the adsorption of ions. Bivalent ions are far more readily adsorbed than monovalent ions, trivalent ions still more readily. If a negatively charged suspensoid or suspension is placed in contact with a source of bivalent cations, these are soon adsorbed, the charge on the colloidal particles is neutralized, and precipitation or coagulation follows. So, too, a positively charged suspension is readily precipitated by bivalent or trivalent anions.

These facts are of course well known and further details will be found in almost any book on colloid chemistry.

Consider now the protoplasmic suspension. We have every reason to believe that it remains stable because of an electric charge. The fact that in living cells generally, bivalent cations are more abundant than <sup>are</sup> bivalent anions, is an indication that the charge on the protoplasmic colloid is positive. Normally, the protoplasmic colloid is in equilibrium both with monovalent and with bivalent cations. If now the percentage of bivalent cations were to be increased, obviously the positive charge, if there is a positive charge, would be increased. On the other hand, if the percentage of bivalent cations were decreased and their place taken by monovalent cations, we should expect a decrease in the charge of the protoplasmic colloid, with perhaps a complete neutralization of charge. Should such a neutralization of charge occur, the protoplasmic suspension would lose its stability, and precipitation or coagulation would be the result.

The point of view that we have been considering is in the main that proposed by HEILBRUNN '23. In the present chapter, no attempt will be made to discuss all the evidence which points toward a positive charge of the protoplasmic suspension. This will be done in the following chapter, which deals with the electrical properties of protoplasm. If, however, we assume a positively charged protoplasmic colloid, we are in a position to understand the actual facts in regard to the effect of sodium and calcium ions on the physical state of the protoplasm.

HEILBRUNN '23 studied the effect of calcium, magnesium, potassium, sodium, and ammonium ions on the protoplasm of the eggs of the sea-urchin *Arbacia*. The eggs were placed in solutions of the chlorides of these metals. In these solutions, the hydrogen ion concentration was carefully controlled, and the concentration of the salt was so adjusted that the solution was isosmotic with the eggs. That is to say, the solutions used caused neither a shrinkage or an increase in volume of the cells. This does not mean that the solutions had the same freezing point. A solution of calcium chloride which produces the same freezing point lowering as a solution of sodium chloride, is demonstrably stronger osmotically with reference to the semipermeable membrane of the *Arbacia* egg. Whether this is due to a greater permeability of the sodium ion or not, does not concern our

present discussion. The point we wish to emphasize is that in the experiments of HEILBRUNN, solutions were used which had no osmotic action on the cell.

In the presence of an increased proportion of the calcium ion, or in isosmotic calcium chloride solutions, the protoplasm of the *Arbacia* egg becomes more fluid. This was clearly shown by centrifuge tests. The magnesium ion produces a similar effect, but its action is not so strong, and the change in viscosity is relatively slight. Potassium, sodium, and ammonium ions cause an increase in the viscosity of the protoplasm. This effect becomes much more pronounced when the eggs are washed relatively free from the sea-water in which they occur normally. Even traces of sea-water markedly retard the coagulative action of potassium, sodium, and ammonium ions. This is apparently due to the greater adsorbability of the bivalent magnesium and calcium ions.

If one arranges the cations in the order of their effect in decreasing the viscosity of the protoplasmic suspension in the interior of *Arbacia* eggs, one obtains the following series:



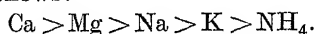
This order is exactly the order of decreasing adsorbability (see books on colloid chemistry). It is thus apparent that the greater the amount of cation adsorbed, the more fluid is the protoplasm of the *Arbacia* egg.

Some of the results of HEILBRUNN '23 have been repeated in subsequent unpublished experiments (compare HEILBRUNN '25c). Particular attention has been paid to the action of calcium. There can be no question but that the calcium ion causes a decrease in the viscosity of the protoplasm. But when *Arbacia* eggs are kept in solutions of isotonic calcium chloride for a long time, a secondary change often occurs. This involves a loss of pigment from the pigment granules, and eventually the egg undergoes a change which by embryologists is usually called cytolysis. As a result of these transformations, the egg becomes coagulated. In later chapters (chapters 13, 14), it will be shown that the loss of pigment from the pigment granules, or the breakdown of these granules in the *Arbacia* egg, always initiates a complicated series of reactions which result in the production of numerous characteristic vacuoles in the protoplasm and a

resultant complete coagulation. Apparently the first stage of this series of reactions depends on a reaction between pigment granules and free calcium. When eggs are placed in calcium solutions, the calcium as it enters the egg is first bound or adsorbed. However, when enough calcium has entered, a reaction with pigment granules occurs and an entire series of changes follows. Curiously enough, it was found that the behavior of the eggs was very different depending on whether or not they were washed in sea-water following removal from the ovary. If eggs were not washed and were placed directly from the ovary into calcium chloride solution, for many hours they showed only a liquefaction of the protoplasm. If however they were thoroughly washed in sea-water and were then placed in an isotonic calcium chloride solution, there was a much greater tendency for the so-called cytolytic changes to occur. The reason for this difference in behavior is not known. Perhaps a difference in permeability is responsible. Perhaps, as HEILBRUNN '25c suggested, a difference in the electric charge of the egg membrane may play a rôle.

The fact remains that the primary action of calcium on *Arbacia* egg protoplasm is to produce a decrease in viscosity. Experiments with the protoplasm of the protozoan *Stentor* gave similar results. In working with *Stentor*, it is not such a simple matter to determine the concentrations of salt solution which are exactly isosmotic with the protoplasm, that is to say cause neither decrease nor increase in the volume of the cell. This is due to the fact that the *Stentor* cell is not spherical and it is therefore impractical to determine its volume. In studying the action of various chlorides on *Stentor* protoplasm, HEILBRUNN '23 used concentrations of magnesium and calcium chloride of slightly lower freezing point than the solutions of sodium and potassium chloride with which they were compared. It is therefore probable that the calcium and magnesium solutions were if anything stronger osmotically than the sodium and potassium solutions. The solutions were so chosen purposely. In general, the more dilute a solution, the more it tends to decrease the viscosity of the protoplasm (see p. 212). In the experiments of HEILBRUNN, it can hardly be assumed that the observed liquefying action of calcium chloride was due to the fact that it was present in lower osmotic concentration.

In the work on *Stentor*, sodium, potassium, lithium, and ammonium chloride were used in m/32 concentration, and magnesium and calcium chloride were used in m/40 concentration. The salts were dissolved in water redistilled from an apparatus made of fused silica and quartz. The pH of the ammonium chloride and lithium chloride solutions was about 6.8, that of the other solutions about 8.0. It was found as a result of centrifuge tests that sodium, potassium, ammonium, and lithium chlorides all caused coagulation of the protoplasm. In solutions of these salts, there was no movement of granules through the *Stentor* cell, whereas in their normal culture fluid or in solutions of magnesium or calcium chloride, the granules moved into one half of the cell when the protozoa were centrifuged. A more rapid coagulative action of the monovalent cations was observed when the cells were first washed free of the culture fluid. This was obviously due to the fact that the culture fluid contained calcium. By making centrifuge tests after exposure to various mixtures of ions, the relative effect of these ions on the viscosity of the protoplasm was measured. It was found that in the order of their liquefying or viscosity-decreasing effect, the order of the ions was as follows:



This order is practically the same as the one found for *Arbacia* protoplasm. The only difference is that the sodium and potassium ions have changed places. In view of the fact that the colloid chemists usually find no decided difference in the adsorption of sodium and potassium ions, this shift is of no importance.

So far, we have considered the action of calcium, sodium, and other cations only on the interior protoplasm. The action of these ions on the surface membrane of the cell is quite different. The primary effect of calcium ions on the cell membrane of the *Arbacia* egg is to make it brittle. It probably decreases in thickness, but this can not be observed under the microscope, for observations at the surfaces of cells are at best uncertain. That the membrane is actually more brittle following exposure to calcium, can be shown by experiments in which the egg is subjected to gradual pressure from above. The eggs are placed in a small drop of sea-water on a slide and then a large cover slip is placed over them. The distance between slide and cover-slip decreases and the eggs are gradually compressed. Under these conditions,

eggs in calcium chloride solution break through the membrane at lower pressures than those in sodium chloride. This is indicated by the fact that the sodium chloride eggs do not break until they have suffered considerably more distortion. The extent of the distortion is determined by measuring the diameter of compressed egg discs. Sodium and ammonium ions in the absence of calcium cause a visible change in the membrane of the *Arbacia* egg. The membrane absorbs water and swells. At the same time it becomes sticky. The presence of calcium prevents this swelling of the membrane produced by sodium and ammonium ions. It should be noted that after long exposure to calcium, the membrane which was at first brittle, also swells and becomes sticky. These effects of various cations on the membrane of the sea-urchin egg were interpreted by HEILBRUNN '23, 25c, as indicating a negative charge of the colloidal particles of the membrane. It was thought that as the percentage of the calcium ion was increased, the charge on the colloidal particles diminished. This would then tend to cause closer approximation of the particles and loss of water. Then on longer exposure to the calcium ion, the charge on the colloidal particles might be reversed, and then the membrane might swell in calcium chloride. Although possibly this explanation may be correct, it is not felt that it rests on as sure ground as the explanation of the effects of the various cations on the interior protoplasm of the cell, and there is indeed some evidence that the colloidal particles of cell membranes are charged positively (see p. 180).

In the previous discussion it has been assumed that the action of calcium, sodium, and other cations was due primarily to an effect on the electric charge of the protoplasmic colloid. If this were the case, it is evident that trivalent cations should have a far more pronounced action than either calcium or magnesium ions. In experiments with inanimate colloids, it has often been shown that trivalent ions are perhaps thousands of times as effective as bivalent cations in altering the charge of suspensions and suspensoids. It became important therefore to discover whether or not trivalent cations had a marked effect on protoplasmic viscosity, and whether this effect was in accord with expectation.

The first experiments with aluminum salts met with no success. The addition of aluminum salts to a solution always

produces a marked acidity, and this acidity is sufficient to cause coagulation of the protoplasm. As a matter of fact, practically all of the aluminum experiments in the literature of plant and animal physiology are open to the objection that the results obtained were due more to the hydrogen ion than to the aluminum ion. Attempts were made to neutralize the acidity due to the hydrolysis of aluminum salt. Such neutralizations, when they were made in the presence of sea-water, always led to an abundant precipitation. The neutral or nearly neutral solutions had no effect on the protoplasm, almost certainly because all of the aluminum ion had been precipitated out of them. Fortunately, it was discovered that if small amounts of aluminum chloride were added to solutions of pure sodium chloride rather than to sea-water, the solution could be brought to a pH of 6 or more without precipitation. A pH of 6 is without any observable effect on the protoplasmic viscosity, so that it is possible to work under these conditions. Why there is no precipitation of the aluminum in the presence of sodium, whereas precipitation occurs in the presence of sea-water, is not certain. Sodium aluminate is soluble and calcium aluminate is very insoluble, but one would hardly expect any formation of aluminates at a pH of 6. Aluminum chloride forms double salts with sodium chloride and perhaps this double salt formation is the important factor. It is hardly necessary to go into the chemical reasons one way or the other. The fact remains that in the presence of only sodium chloride, aluminum can be kept in solution without any strong acidity. This fact makes it possible to work with aluminum salts with the knowledge that one is studying only the effect of the aluminum ion and not the combined effect of aluminum and hydrogen ions.

Once the method of work was discovered, results were easy to obtain. It was soon found that very dilute solutions of aluminum chloride produced a very marked effect on the viscosity of the protoplasm of the *Arbacia* egg. Even a  $m/25,000$  solution (in isotonic sodium chloride solution) was sufficient to cause a very pronounced decrease in the viscosity of the protoplasm. Solutions of cerium chloride behaved in the same way as those of aluminum chloride, except that somewhat higher concentrations had to be used to produce the same result. The primary effect of aluminum and cerium is thus to cause a decrease in the viscosity of the protoplasmic suspension.

However, after long exposure, especially if the eggs have been washed in sea-water before being placed in contact with the solutions containing aluminum or cerium, a secondary effect occurs. This is first indicated by a change in the appearance of the outer membrane of the egg. This membrane is at first brittle, but later it may absorb water, swell and become sticky. As soon as this happens, there is usually a coagulation within the egg interior.

The account here given follows in general the preliminary note of HEILBRUNN '25c. Perhaps at some time in the future a more complete record of the experiments will be published. From what has been said, however, it must be clear that the action of trivalent cations is in accord with the proposed theory. The first effect is certainly a decrease in viscosity. As the trivalent ions enter the protoplasm slowly, they are probably adsorbed at the surfaces of the dispersed phase of the suspension, and this results in an increase in electric charge and a decrease in viscosity. But when the outer membrane of the egg is changed, the trivalent ions may enter too rapidly and they may then have an effect on the proteins of the dispersion medium.

The experiments of HEILBRUNN show clearly that in *Arbacia* protoplasm and in *Stentor*, various cations act in a manner exactly comparable to the way they act on an inanimate suspension. There are complications, it is true, but the main facts seem plain enough. In general it is our claim, that aside from secondary reactions, calcium and magnesium tend to cause a decrease in protoplasmic viscosity as compared with potassium, sodium, and ammonium. We shall now proceed to see whether or not this point of view finds support in the literature.

Perhaps the most striking evidence that sodium and potassium ions cause a coagulation of the protoplasmic suspension is found in the observations of REZNIKOFF and CHAMBERS '25 and CHAMBERS and REZNIKOFF '26. These authors immersed amebae in solutions of sodium chloride and potassium chloride and they found that within relatively few minutes the granules of the ameba protoplasm clumped together and sank through the cell. The picture that is described and figured by these authors apparently is a typical coagulation picture, and yet they interpret their results as showing a decreased viscosity or a liquefaction of the protoplasm. Surely, when particles of a



suspension aggregate and then fall, this is not due to a change in the viscosity of the dispersion medium. It is due to the fact, that in accordance with STOKES' law, the speed of fall of a spherical body through a fluid varies as the square of the radius. As the particles of an inanimate suspension, or as the granules of ameba come together, they form a larger aggregate and this because of its greater size, falls rapidly. From STOKES' law it can be shown that, on any assumption as to the magnitude of the absolute viscosity of the protoplasm of the ameba, on any reasonable assumption as to the magnitude of the specific gravity of the granules, these granules of the ameba cell could not possibly fall at even approximately the rate observed by CHAMBERS and REZNIKOFF, if they fell singly. Indeed these authors describe a clumping of granules, and the clump remains at the bottom of the cell for an indefinite period of time. In all the literature on the colloid chemistry of protoplasm there is perhaps no clearer case of coagulation.

That sodium and potassium produce coagulation of ameba protoplasm is also indicated by the fact that these ions are far more toxic than the ions of calcium and magnesium. Thus, according to CHAMBERS and REZNIKOFF '25, amebae will live for 24 hours in m/6.5 magnesium chloride and for about 5 hours in m/6.5 calcium chloride, whereas they die within an hour in m/6.5 sodium chloride, and they die within an hour in solutions of KCl as dilute as m/104. Whenever death occurs in solutions of sodium or potassium salts, this death is accompanied by a clumping and sinking of granules. Amebae will live for three days or more in solutions of m/13  $MgCl_2$  or m/26  $CaCl_2$ , so that it seems probable that in solutions weak enough to be without osmotic effect, ameba will live indefinitely in pure calcium or magnesium solutions.

REZNIKOFF and CHAMBERS and CHAMBERS and REZNIKOFF also describe experiments in which they injected solutions of calcium, sodium, and other salts into the protoplasm of ameba. In these experiments too, the sodium and potassium ions caused a sinking and clumping of granules. Evidently under these conditions also, the sodium and potassium ions produce coagulation. CHAMBERS and REZNIKOFF are of the opinion that the injection of calcium into the ameba protoplasm causes a coagulation. This may very possibly be true. Ordinarily when cal-

cium enters a cell, it enters slowly and it is adsorbed as soon as it enters. The injection of calcium salt into a cell would expose the protoplasm to free calcium ions. In a later chapter (chap. 14), it will be shown that free calcium initiates a complicated series of reactions in protoplasm, the end result of which is a coagulation\*.

The observations of CHOLODNY '23 are similar to those of CHAMBERS and REZNIKOFF and they show very clearly that sodium and potassium ions have a coagulative action, and that further this coagulative action is prevented by the presence of calcium ions. CHOLODNY studied the root hairs of *Trianea bogotensis*, an aquatic plant. He placed pieces of roots in glass dishes and found that they could live for a long time (over 24 hours) in water doubly distilled from glass. They also live as long as this in solutions of calcium chloride or other calcium salts, and this is not due to a failure of the calcium to enter the cells (see below). But when the roots are placed in solutions of sodium or potassium salts, coagulative changes can be seen to occur in the protoplasm, and death follows. CHOLODNY's description of these changes is very clear and it is here given in his own words. "Wenn wir aber die Wurzelhaare in KCl-Lösung oder in die Lösung eines anderen K-Salzes bringen, so können wir nach einer gewissen Zeit (die von der Salzkonzentration abhängt aber stets schneller als in einer Stunde) folgende interessante Erscheinung beobachten. Die Plasmbewegung wird immer langsamer, als ob

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\* MAST '28 reports experiments on the effect of salts on ameba. His results do not agree 154 fully with those of CHAMBERS and REZNIKOFF. BRINLEY '28b has studied the effect of salts on the viscosity of ameba protoplasm. He used the amplitude of Brownian movement as an index of viscosity, and observations were made almost immediately, within 2 minutes after entrance of the ameba into the solutions (personal communication). In sodium chloride the ameba appeared to increase in volume, owing to a dissolving effect on the plasmalemma (personal communication). In calcium and magnesium chloride there was probably a volume decrease, for the solutions used were apparently hypertonic. When the ameba volume increases there is more room for Brownian movement, and when it decreases there is less. This is probably an explanation of BRINLEY's results. It would be hard to assume that the salts could diffuse far into an ameba within 2 minutes. Note added to proof.

dies durch Viskositätsvergrößerung verursacht wäre, und zugleich ist an der Haarspitze (selten an anderen Stellen) eine Anhäufung des Protoplasmas zu beobachten, welches hier bald ein festes Gerinnsel bildet. Dieses Plasmagerinnsel hat gewöhnlich so große Dimensionen, daß es sogar mit Hilfe einer Lupe zu sehen ist, wobei es als trübweißes Klümpchen, welches den peripheren Teil der Zelle ausfüllt, erscheint. Diese Erscheinung ist als allmähliche Plasmagerinnung zu betrachten: bald darauf folgt der Tod. In n/20 KCl-Lösungen starben alle Haare gewöhnlich nach 30—40 Minuten ab." No one reading CHOLODNY's description could doubt that the potassium ions cause a coagulation of the protoplasm of *Trianea* root hairs. If a little calcium chloride is added, coagulative phenomena are never seen. Furthermore, if a root hair in which the potassium ion has already begun to produce its effect is placed in a solution containing calcium, either in a pure solution of a calcium salt, or in a solution of potassium chloride plus calcium chloride, then after a time there is a liquefaction of the coagulum, and a renewal of protoplasmic streaming. The coagulum does not always disappear wholly, at times a small part of it remains. From CHOLODNY's account it is certain that the potassium ion produces a coagulation within the protoplasm of the root hairs of *Trianea*. CHOLODNY states that sodium, lithium, and ammonium behave in similar fashion. Potassium and ammonium have a more pronounced effect than sodium. It should be particularly noted that even after coagulation has been initiated by the potassium ion, this may be reversed by immersion of the roots in a solution containing calcium. Obviously the calcium must enter the cell to produce this effect, at any rate this is certainly highly probable.

Another clear case in which the sodium ion causes coagulation and the calcium ion liquefaction is provided by the experiments of WEBER '24d. WEBER treated filaments of *Spirogyra orthospira* with solutions of calcium and with a solution of sodium chloride for long periods, and then tested the protoplasmic viscosity both by the centrifuge method and by the plasmolysis form method. Both methods gave consistent results, and it is evident that in this type of protoplasm the sodium ion causes coagulation, the calcium ion liquefaction. WEBER's experiments are recorded in the following table.

Action of salts of calcium and sodium on the protoplasmic viscosity of *Spirogyra* (WEBER '24d)

Salt. All salts in m/10 solution	Length of exposure	Type of plasmolysis	Readiness with which chloroplasts move on centrifugal treatment
$\text{Ca}(\text{NO}_3)_2$	20 hours	convex	chloroplasts move readily
$\text{Ca}(\text{NO}_3)_2$	4 days	convex	chloroplasts move readily
$\text{Ca}(\text{NO}_3)_2$	5 days	convex	chloroplasts move readily
$\text{CaCl}_2$	3 days	convex	chloroplasts move readily
$\text{CaCl}_2$	8 days	convex	chloroplasts move very readily
$\text{NaCl}$	30 hours	"Krampfplasmolyse"	chloroplasts move with great difficulty
Tap water	controls	angular ("eckig")	chloroplasts move with difficulty

The table shows that calcium solutions cause a decrease in the viscosity of the protoplasm of the treated cells as compared with that of the controls in tap water, and that a sodium chloride solution has the opposite effect. It should be noted that the calcium chloride solutions were stronger osmotically than the sodium chloride solution, but this would tend to make the protoplasmic viscosity higher not lower, as was actually found to be the case (for effect of hypotonicity see p. 212).

In his paper, WEBER cites HANSTEEN CRANNER '19 as having shown that plasmolysis by a potassium chloride solution is uneven or angular, whereas when cells are plasmolyzed by a calcium chloride solution, the boundary of the cells is smooth, that is to say the plasmolysis is convex. HANSTEEN CRANNER worked with the epidermis cells of the scales of the onion. His figures are very beautiful and show clearly two different types of plasmolysis. CHOLODNY '24 also found in onion cells an irregular or "eckig" plasmolysis following plasmolysis with sodium chloride solutions. But WEIS '25 and PRÁT '26 working with the same material, but apparently unaware of the older results, both found that when the onion cells were plasmolyzed in ammonium, potassium, or sodium chloride solutions, the surface of the cell was smoother, i. e. more convex than when plasmolysis occurred in calcium chloride solutions. This divergence of results is probably not hard to explain. HANSTEEN CRANNER compared

normal solutions of potassium and calcium chloride. In his experiment, the potassium chloride solution was the stronger osmotically. WEIS on the other hand used solutions of equal molarity, and in his experiments the calcium solution was stronger than the solutions of sodium, potassium, and ammonium chloride. PRÁT gives the concentrations of his solutions in percents, but apparently he too used stronger solutions of calcium chloride\*.

As a matter of fact, all of these experiments in which the cells are placed directly in the plasmolyzing solutions are probably of little value as a source of information regarding the viscosity of the protoplasm in the interior of the cell. The cell plasmolyzes before the calcium and sodium ions have time to penetrate into the interior and produce a result. It is to be noted that in WEBER's experiments he treated the *Spirogyra* cells a long time with the salt solutions before he subjected them to plasmolysis.

The experiments of CHAMBERS and REZNIKOFF, those of CHOLODNY, and those of WEBER, all support HEILBRUNN's claim that calcium ions cause decreased viscosity of protoplasm, and that sodium, potassium, and ammonium ions have the opposite effect. There is thus direct evidence, not only for sea-urchin eggs and for *Stentor* protoplasm, but also for the protoplasm of *Ameba*, for the root hairs of *Trianea bogotensis*, and for the protoplasm of *Spirogyra*, that this view is correct.

There is also some indirect evidence. SCARTH '24a, 24b, described some visible changes in *Spirogyra* cells following treatment with various bivalent and trivalent cations. These he regards as indicative of an increase in fluidity. Such an interpretation would accord very well with the point of view expressed in the preceding pages, but unfortunately SCARTH's reasoning is not very convincing. In the first of his two papers, SCARTH mentions the fact that "Ba was found to produce rapid streaming of protoplasm in the case of the onion", and he also cites SEIFRIZ '22, who found that strontium stimulated protoplasmic streaming in *Elodea* cells. The fact that these bivalent ions favor a rapid rate of protoplasmic streaming is consistent

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\* Tatsächlich spielt innerhalb eines bestimmten Konzentrationsbereiches die Konzentration des Plasmolytikums eine entscheidende Rolle für das Zustandekommen der Plasmolyseform. Note written by F. WEBER.

with the idea that they cause a decrease in protoplasmic viscosity, although of course the effect of the ions may be on the motive force of the streaming.

Unfortunately there have not been many experiments on the effects of various cations on protoplasmic streaming. The experiments of SEIFRIZ '23b are not entirely satisfactory, for he compares the action of 0.128 M  $\text{CaCl}_2$  with that of 0.128 M  $\text{NaCl}$ , and these solutions are not isosmotic as he believes them to be.

KAHO '21b, 24a, has performed many interesting experiments on the effects of various salts on plant cells. In these experiments the salts are used in relatively high concentration, and their toxic action is compared by noting how soon the cells are killed. In order to determine the death point, KAHÖ treats the cells with a plasmolytic agent. If they plasmolyze, they are considered to be still alive; if they fail to plasmolyze, they are dead. It seems probable that the failure of a cell to plasmolyze is the result of coagulation of its protoplasm. We can therefore interpret KAHÖ's results in this fashion. KAHÖ '21b found that when *Tradescantia* cells were exposed to 0.2 n solutions of potassium, ammonium, sodium, strontium, magnesium, barium, and calcium chloride, death occurred much more rapidly in the solutions of salts with monovalent cations, than in those with bivalent cations. Thus in the potassium chloride solution, almost half of the cells were dead after 4 hours, whereas even after 20 hours, all of the cells exposed to the calcium chloride solution were still alive. It should be pointed out however, that the solutions of salts with bivalent cations are weaker osmotically, and that this may be an important factor. KAHÖ believes his results are due to differences of permeability, and this may very well be true. An alternative interpretation is that the bivalent cations do not cause coagulation, whereas the salts of the monovalent cations do.

In this connection it might be worth mentioning that since 1864 it has been known that the roots of bean plants can live indefinitely in solutions of pure calcium chloride, although they are killed or injured in weaker solutions of various other salts (WOLF '64). In more recent years, this knowledge has been extended for many other types of roots by HANSTEEN CRANNER '10, '14, '22. This author finds that calcium chloride is non-toxic even when it is present in what he considers the relatively strong concentration of n/50. Obviously in these experiments the cal-

cium enters the cells of the roots, for the plants live for indefinite periods in the solutions of calcium salts\*.

In view of the work of HANSTEEN CRANNER and of the older work of WOLF and others, it is rather surprising to find that ADDOMS claims that the calcium ion has a toxic effect on the root hairs of the wheat plant after only ten minutes exposure. HANSTEEN CRANNER, WOLF '64, BOEHM '75, all found that the roots of various plants could live for indefinite periods when exposed to calcium. ADDOMS after 10 minutes finds a toxic effect, which is indicated by a coagulation of the protoplasm of the root hair. The explanation is not far to seek. ADDOMS used a  $n/10$  solution, and this was evidently too strong osmotically. This explains why the solutions exerted an action within 10 minutes. One could hardly expect calcium ions to diffuse deeply into the protoplasm within 10 minutes. ADDOMS also finds coagulative changes following exposure to  $n/10$  solutions of sodium, potassium, magnesium, zinc, and aluminum salts. In all of these cases the effect may be due to the osmotic action of the solution. However, in the case of the zinc and aluminum solutions, the high concentration of hydrogen ions probably played a part.

The work that we have considered so far, with the exception of that of ADDOMS, has favored the view that calcium ions tend to produce liquefaction or decreased viscosity of the protoplasm, and that sodium and potassium ions have the opposite effect. We shall now consider two sets of experiments which do not fit in with this conception.

ERTSCH and JOCHIMS '27a studied the appearance of nerve fibers of the frog in the darkfield, using a cardioid condensor. The work seems to have been very carefully done, and the photographs are excellent. Unfortunately the nerve fiber is surrounded by two sheaths, and the outer of these sheaths gives a very bright image under darkfield illumination. Under such conditions, observation of the interior of the fiber can scarcely be very accurate. One need only be reminded of the warnings of GAIDU-

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\* For other experiments on the effect of various ions on the growth of plant roots, and for many useful references to literature, see MEVIUS '27. MEVIUS studied the effect of different salts at various hydrogen ion concentrations.

KOV '10 and PRICE '14 against the use of such material for dark-field study (see p. 38). SHIWAGO '26 found the nucleus of leucocytes optically empty in darkfield, whereas structures were visible in it in the brightfield. This he rightly attributes to the brightness of the nuclear surface which renders observation of the interior impossible. It is readily understood why ETTISCH and JOCHIMS find the interior of normal nerve fiber optically empty in spite of the fact that there are doubtless visible colloidal micellae there, just as there are in all other types of protoplasm. The appearance of brightness within the fiber may be due either to the appearance of a new phase, or to a change in the optical relations which permit more light to enter the fiber. ETTISCH and JOCHIMS report that in the presence of calcium salts, the interior of the fiber becomes brighter, and they regard this as evidence of coagulation. But they state, and their pictures show, that the calcium ion has a marked effect on at least one of the sheaths of the nerve fiber, and the increased brightness might be due to this factor alone.

Finally we shall consider the peculiar results of TIMMEL '27. This author studied rather a wide variety of plants, but in particular *Caltha palustris* and *Monarda didyma*. He studied stems and sections of stems, and determined viscosity by the centrifuge method. When the stems are centrifuged, ordinarily there is no movement of chloroplasts, but when the cells are exposed for a short time (5 minutes) to a half molar solution of potassium chloride, then the chloroplasts move through the cell when the plant tissues are centrifuged. Potassium nitrate seems to produce the strongest effect, potassium chloride and sulphate have a less pronounced action. Ions of sodium, lithium, magnesium, barium, and calcium, have no noticeable influence. It must be admitted that these results are very unexpected and one is at a loss for an explanation. One would expect that if potassium had a liquefying action, sodium would likewise have such an action.

Let us examine TIMMEL's experimental evidence a little more closely. In the first place, it is noteworthy that he used solutions which plasmolyzed the cells. He states however that solutions which did not cause plasmolysis, 0.4 n or weaker, also have the same effect. How much weaker the solutions could be and still prove effective, TIMMEL does not state. But he does state that 0.1 n potassium nitrate solutions are ineffective. If the action of the



salt is due solely to an effect of the potassium ion, one is tempted to inquire why these weaker solutions do not alter the viscosity after sufficiently long exposure. TIMMEL exposed the tissues to a 0.1n concentration of the salt for 24 hours. After 24 hours exposure to 0.1n KCl, probably more potassium ion would penetrate the cells, than after 5 minutes exposure to 0.5 n KCl. Another interesting fact regarding TIMMEL's experiments is that in some months of the year the potassium is without an effect unless it is combined with another plasmolytic agent such as cane sugar.

From these considerations it seems probable that the peculiar results of TIMMEL are due as much to the abnormally high concentration of the salt as to the specific effect of the potassium ion. But it is nevertheless very remarkable that hypertonic solutions of potassium salts can cause a decrease in viscosity, for in protoplasm generally, decrease in water content of the cell is accompanied by increase in viscosity.

It seems certain that TIMMEL's results are peculiar to certain types of protoplasm, and can not hold generally. He found that relatively concentrated solutions of potassium salts, but not sodium salts, made it possible for the chromatophores of certain plant cells to be more readily moved by centrifugal force, when the experiments were performed at some seasons of the year rather than others. Perhaps the potassium salts react in some peculiar way with constituents of the plant protoplasm. But there is also another type of explanation which is possible.

From TIMMEL's single figure, it seems that the chromatophores of the cells he studied are close to the outer boundary of the cell. Perhaps in this position they may under certain conditions be anchored to this membranous boundary. If large granules or chromatophores are anchored to the outer membrane of a cell, one could hardly expect them to be moved by centrifugal force. Thus HEILBRUNN '20a found that in the sea-urchin egg, at times when the protoplasm in general was of low viscosity, the pigment granules might assume a position close to the outer membrane and there resist movement following centrifugal treatment. In plant cells also, a slight movement of chromatophores away from the outer boundary of the cell might make them much more readily movable by centrifugal force.

In view of the fact that the size, the shape, and the position of the chromatophores within plant cells are profoundly modified

by all sorts of external conditions, it is perhaps unsafe to use the movement of chromatophores or their failure to move when cells are centrifuged, as any index of the viscosity of the protoplasm, unless at least one can be certain that the conditions of the experiment have no effect on the position of the chromatophores within the cell. SENN '08 has gathered together a great mass of evidence concerning the movement of chromatophores under different conditions. It is interesting to note that KÜSTER, in whose laboratory TIMMEL'S work was done, himself reports movement of chromatophores away from the outer wall of plant cells under the influence of solutions more concentrated than the normal environment, but not concentrated enough to cause plasmolysis (see KÜSTER '05).

In the discussion up to this point, we have considered only those authors who have attempted a physical study of the action of salts on protoplasm. In the literature, and particularly in the older literature, many writers expressed opinions as to the effect one salt or another might have on the colloidal state of the protoplasm. Some of these opinions were based on guesses, some on analogy, and others were arrived at after the authors had looked at cells immersed in various salt solutions. Thus GREELEY '04 from morphological appearances attempted to distinguish coagulation and liquefaction in paramecium after this protozoan had been exposed to different solutions of salts. The figures GREELEY draws are not very convincing, nor do his conclusions appear very probable. Thus for instance he is of the opinion that m/40 KCl coagulates the protoplasm, whereas to m/40 NaCl he ascribes a liquefying effect.

SPEK '20 experimented on the action of various salts on paramecium. He found that LiBr, LiCl, and KSCN caused the paramecia to increase in volume and he attributed this effect to a direct swelling action on the colloids of the protoplasm. By actual viscosity measurement, it can be shown that lithium salts cause a coagulation, that is to say a great increase in viscosity in the protoplasm of the protozoan *Stentor* (see above), and also in the protoplasm of paramecium (unpublished experiment of Mr. L. G. BARTH). Very frequently the increase in volume of cells is accompanied by coagulation.

In 1921, SPEK studied the effect of various salts on the protoplasm of *Actinosphaerium*, and he describes certain types of

morphological change which he regards as significant of colloidal change. In this as in all of his papers, SPEK makes the error of assuming that equimolar solutions of sodium and calcium chloride are isosmotic. In observations on the protozoan parasite *Opalina*, SPEK '23a finds that the addition of rather large mounts of 0.3 M calcium chloride causes shrinkage of the *Opalina* cells, whereas the addition of 0.3 M sodium or potassium chloride apparently has no such effect. Quite apart from any specific action of ions, it should be clearly understood that a 0.3 M calcium chloride solution is almost one and a half times as strong osmotically as a sodium chloride solution of the same molar concentration. If one were to assume a relative impermeability of the calcium ion, as SPEK does, the calcium chloride solution would be even stronger osmotically as compared with the sodium chloride solution. It is not probable that all of the effects described by SPEK are due to the osmotic differences of the solutions he used, but it hardly seems worth while to attempt to interpret his morphological data, complicated as they are by his misunderstanding of osmotic relationships.

GIERSBERG's studies of the action of salts on the protoplasm of ameba are in the same category as those of SPEK. He too compares solutions of equimolar strength. In one of the two tables in which he compares the action of sodium and calcium chlorides, he finds that m/20 calcium chloride leaves the protoplasm perfectly normal for 12 days, whereas m/20 sodium chloride causes the production of granulations and various other pathological changes in the protoplasm. In the other of the two tables, m/20 calcium chloride is shown to cause a rounding up of the protoplasm and death within two days. GIERSBERG's comment is that one could hardly expect to get the same result each time! According to REZNIKOFF and CHAMBERS '25, amebae can live for at least three days in m/26 calcium chloride.

In resumé, it may be stated that the work of HELBRUNN, CHAMBERS and REZNIKOFF, CHOLODNY, and WEBER all shows clearly that for various types of protoplasm, sodium, potassium, and ammonium ions cause coagulative changes, and that these changes are prevented by the ions of magnesium and calcium, which tend to have the opposite effect. The work of these authors rests on a safe, sure basis and can hardly be challenged. In addition, there is some indirect evidence that is in harmony with the above

thesis. On the other hand, there is apparently no reliable experimental evidence that is in conflict with it. In the future, it may perhaps be shown that some types of protoplasm behave very differently from the protoplasm of sea-urchin eggs, *Stentor*, *Trianea*, ameba, and *Spirogyra*. This is quite possible, for it may be true that some types of living material have colloidal particles charged differently from those of the cells we have considered. But at the present time there is no direct evidence that this is so.

It should be remembered that our statement regarding the effect of sodium, calcium, and other similar ions on the protoplasm is only true for the protoplasm of the interior of the cell. Moreover it should also be noted that the primary effect of these ions may be complicated by secondary effects, such as we have described for calcium in the case of the sea-urchin egg.

There has been very little study of the action of anions on the colloidal properties of living protoplasm. In the sea-urchin egg such study would not be easy. Various anions, iodides for example, cause changes in the osmotic membrane of the egg, and these changes result in a large increase in the volume of the cell. When sea-urchin eggs are placed in solutions of sodium iodide which at first appear to be isosmotic, after a time the cell takes up a considerable quantity of water, and the colloidal changes of the protoplasm are due as much to this water intake as to any specific effect of the anion.

KAHO '21b exposed sections of *Tradescantia* leaves and of red cabbage to various salts of sodium, potassium, and ammonium. Not only the cations, but the anions as well differed in their effect on the cells. KAHO studied the time required for the cells to be killed in the different solutions, using as an index of death, the failure of the cells to plasmolyze when treated with hypertonic solutions. Such a failure to plasmolyze is probably, as KAHO supposes, evidence of coagulation. KAHO found that the order in which the various anions favored coagulation was the following:

$\text{CNS} > \text{I} > \text{Br} > \text{NO}_3 > \text{C}_2\text{H}_3\text{O}_2 > \text{Cl} > \text{Tartrate} > \text{Citrate} > \text{SO}_4$ .  
In his experiments, KAHO compared equinormal solutions, so that the solutions of tartrate, citrate, and sulphate were much weaker osmotically than the other solutions. This may be a factor to be

considered, for apparently KAHO generally used hypertonic solutions. It is understandable, therefore, why tartrate, citrate, and sulphate are less toxic than the other salts. The salts of monovalent anions were used in approximately isosmotic concentrations, so that the results with these salts are more interesting. From KAHO's results it seems possible to conclude that the sulphocyanate ion and the iodide ion have a much stronger coagulative action than the chloride ion. This is readily understandable on the theory that we have proposed. The sulphocyanate ion and the iodide ion are known to be more readily adsorbed than the chloride ion and hence they would have a greater effect in neutralizing the positive charge of the protoplasmic suspension. KAHO himself believes his results are explainable on the basis of differential permeability, the ions which penetrate most rapidly causing death or coagulation soonest. This seems a perfectly possible alternative interpretation.

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## CHAPTER X

# THE ELECTRIC CHARGES OF PROTOPLASM

In any colloid the electric charge on the colloid particles or micellae is of great importance. More than anything else, it is this charge that keeps the particles from coalescing. When the charge is neutralized, the particles form larger aggregates, and these settle out of solution. Of the two main types of colloidal solutions, the lyophobic colloids are even more dependent for their stability on the electric charge of the dispersed particles than are the lyophilic colloids.

On the basis of its colloidal behavior, protoplasm really has more in common with lyophobic colloids than with lyophilic colloids. This is readily understandable when we remember that protoplasm is actually a suspension (see p. 20). Obviously one of the most important problems in the study of the colloid chemistry of protoplasm is the determination of the sign of the electric charge on the colloidal particles of the protoplasm.

A colloidal solution may have particles with a positive charge or with a negative charge. For the colloid chemist who deals with inanimate colloids, it is usually a simple matter to decide whether the colloid he is studying is of the one type or the other. The colloidal solution is placed between suitable electrodes, and from the migration of the particles either to the anode or to the cathode, the sign of the charge can be determined. For the biologist the problem is not so simple. If he attempts to study the cataphoresis of living protoplasm, he must follow the movements of the colloidal particles not in any container of his choice, but within the cell. Every student of cataphoresis knows of the complicating effect of the wall of the cataphoresis chamber. If colloidal particles travel in one direction through the center of a cataphoresis chamber, they may travel in the opposite direction along the walls, for there they are carried by the dispersion medium which can move in a direction opposite to that of the colloidal

particles. If the wall of a living cell is charged electrically, as it probably is, there would be a tendency for dispersion medium to flow along it. But this is not the only difficulty, or even the chief difficulty. When one passes an electric current through an inanimate colloid, there is no coagulation or other disturbance except in the immediate vicinity of the electrodes. But when even relatively weak currents are sent through living cells, there is a practically instantaneous coagulation of the entire protoplasm (see p. 131). Such a coagulation would of course prevent any cataphoretic movement of the colloidal particles of the protoplasm, or it might very well bring about a reversal of the normal charge.

From what has been said, it is obvious that any evidence regarding the cataphoretic migration of protoplasmic granules or colloidal particles must be viewed critically and carefully.

Fortunately the colloid chemist also has a second method which makes it possible for him to determine the electric charge of a colloid without the use of an electric current. This second method is particularly applicable to suspensoids and suspensions. It can therefore be used for the protoplasmic suspension. The principle of the method is simple enough. In recent colloid chemical theory it is generally assumed that the charge on the colloidal particles is primarily due to adsorbed ions. It is well known that bivalent ions are adsorbed more readily than monovalent ions, and that trivalent ions are adsorbed far more readily than bivalent ions. If then bivalent or trivalent cations are added to a colloid, they will tend to neutralize the charge on the colloidal particles and produce precipitation if this charge was originally negative, whereas if the charge was originally positive, they will have no such effect. On the other hand bivalent and trivalent anions tend to precipitate positively charged colloids. For details concerning the use of this method, various books on colloid chemistry may be consulted.

Analyses of living cells typically show an excess of bivalent cations over bivalent anions (see p. 25). Thus protoplasm usually contains more calcium and magnesium ion than it does sulphate ion. This is in itself an indication that the protoplasmic suspension bears a positive charge. Much stronger evidence however is obtained when the protoplasm is exposed to the action of various ions. Normally in the cell there is a mixture of monovalent and bi-

valent cations. If we expose cells to pure solutions of salts with monovalent cations, i. e. sodium or potassium or ammonium, the amount of bivalent cation adsorbed on the surfaces of the colloidal particles or granules of the protoplasm is decreased. If the charge on the protoplasm were negative, this would have no effect or would favor liquefaction. As a matter of fact, the sodium, potassium, and ammonium ions produce a coagulation of the protoplasmic suspension in all cases in which accurate data have been obtained (see previous chapter). Hence, from this fact, and from the fact that the addition of calcium or magnesium ions produces liquefaction as a primary effect, we may conclude that the charge on the granules of the protoplasmic suspension is positive. Just as in the case of inanimate colloids, the action of trivalent ions is far more potent than that of bivalent ions, so too in protoplasm the trivalent ions aluminum and cerium are extremely effective in producing a liquefaction of the protoplasm (see p. 151).

We are thus led to the conclusion that the colloidal particles of the protoplasmic suspension, or the granules, bear a positive charge. This is an extremely important conclusion, and we can hardly be content with one line of evidence. The protoplasmic colloid is far more complicated than any inanimate colloid, and the chances of errors of interpretation are proportionately so much greater. In the pages that follow, therefore, we shall attempt to see whether or not the assumption of a positive electric charge on the colloidal elements of the protoplasm is in harmony with various known facts of cell physiology.

In the first place we shall try to assemble all the known experiments on the cataphoresis of the protoplasm in the interior of living cells. The literature on this subject is more extensive than is generally thought. At first sight it appears inconsistent, for whereas some authors have described a migration of protoplasmic particles toward the cathode, others have described a migration toward the anode. Fortunately, a critical survey of the literature seems to indicate that there is no contradiction between the results of the different investigators.

The study of the cataphoresis of protoplasm is as old as the study of cataphoresis itself. In what appears to have been the first clear-cut description of cataphoresis, JÜRGENSEN '60 studied the migration of carmine particles and of lycopodium powder in an electric field. In the same paper, JÜRGENSEN also reported



some experiments on the effect of an electric current on the protoplasm of the leaves of *Vallisneria*. In his description of his results, JÜRGENSEN states that the current first killed the cells, and that then the protoplasm migrated to the anode. This sort of experiment has been frequently performed by subsequent investigators, and it seems certain that after death the protoplasmic colloids have a negative charge.

But of course we are more interested in the charge of the protoplasmic colloid during life. The first description of cataphoresis of living protoplasm is that of KÜHNE '64. On page 79 of his classic monograph, KÜHNE describes an experiment in which a constant electric current from six small Grove cells was passed through some myxomycete protoplasm. The electrodes were broad platinum plates, and they were 4 mm. apart. As soon as the current entered the protoplasm, it caused a movement of granules toward the cathode.

“Im Momente, wo ich die Kette schloß, fand eine ruckweise eintretende Beschleunigung der Körnchenströmung statt, welche vom positiven zum negativen Pole gerichtet war, während die entgegengesetzt fließende für einen Augenblick stillstand oder auch etwas zurückwich. Umkehrungen der Strömung von irgendwelcher längeren Dauer konnte ich von dem konstanten Strom nicht erreichen; ich überzeugte mich aber durch rasches Umwerfen einer in den Kreis geschalteten POHLschen Wippe davon, daß die Erscheinung auf das JÜRGENSSSENSche Phänomen zurückzuführen sei. Jede Bewegung der Körnchen, die sich überhaupt abhängig zeigte von der Richtung des elektrischen Stromes, verlief vom negativen zum positiven Pol. Ohne Zweifel haben wir es hier wirklich mit dem JÜRGENSSSENSchen Phänomen zu tun, denn eine entsprechende Kontraktion auf dem einen, bald auf dem anderen Ende der Myxomycete in Abhängigkeit von den Ein- und Austrittstabellen des E.-Stromes konnte nicht beobachtet werden.”

In the above quotation, the reference is doubtless to JÜRGENSEN '60, in spite of the difference in spelling, so that “JÜRGENSSSENSchen Phänomen” is equivalent to cataphoresis. In one respect, the quotation from KÜHNE is ambiguous. After stating clearly enough that the electric current caused an acceleration in the movement of granules toward the cathode and a retardation of the granules moving naturally in the opposite direction, KÜHNE

states that in order to make sure that the effect was due to the electric current he reversed the current and then found a movement of granules toward the positive pole. There is only one way to interpret this so as to make sense. It seems certain that after KÜHNE reversed the current, the movement of granules was toward the pole which was *originally* positive.

In the further discussion of his experiment, KÜHNE is careful to note that there was no contraction of the protoplasm at the points where the current passed in and out. There was, it is true, a slight contraction at the make and break of the current, but this was equally evident at both positive and negative poles, and was not confined to the parts immediately in contact with the electrodes. KÜHNE notes that the effect of the constant electric current on the movement of granules does not last; his electrodes are polarizable, and gradually the diffusion of substances formed at the electrodes interferes with the phenomenon.

KÜHNE seems to have had a clear understanding of the conditions of his experiment, and it is probable that he actually did see a cataphoretic movement of the protoplasmic granules toward the negative electrode, that is to say, the cathode.

In 1876, VELTEN ('76c) wrote a paper which is primarily concerned with the action of electric currents on protoplasmic streaming in various types of plant cells. In the summary of this paper appears the following statement: "Bei starken elektrischen Strömen sammelt sich das Protoplasma vorzugsweise gern an der positiven und negativen Polen zugekehrten Zellwand in Form von Platten oder ellipsoidischen Körpern an. (Die Stromestärken, welche eine Wanderung des Zellinhaltes nach dem positiven Pol hervorrufen, übersteigen die Ströme, die hier als 'sehr stark' bezeichnet werden.)" Velten then goes on to say that the terms strong, weak, etc., are only in relation to the effects of the electric current on the protoplasm. VELTEN found that the protoplasm might wander either to the anode or the cathode, but that it was the very strong currents that caused a movement to the anode. From this one can infer that the weaker currents caused a migration toward the cathode.

The observations of VELTEN are very interesting, for if the interpretation we have taken is the correct one, the results of VELTEN are exactly like those reported many years later by HARDY.

In a short paper of only four pages, HARDY '13 brings forward what is apparently the most clear-cut evidence regarding the cataphoretic migration and the electric charge of the internal protoplasm of living cells. HARDY placed onion root-tips horizontally between non-polarizable electrodes, the final lead to the tissue being through some of the fluid in which the root had been growing. A field having a mean value of 5 to 20 volts per centimeter was established for from 2 to 10 minutes, after which time the root was instantly fixed in a solution of acetic acid and absolute alcohol. HARDY did not attempt to determine the strength of the field to which the living tissue was really exposed, for there is

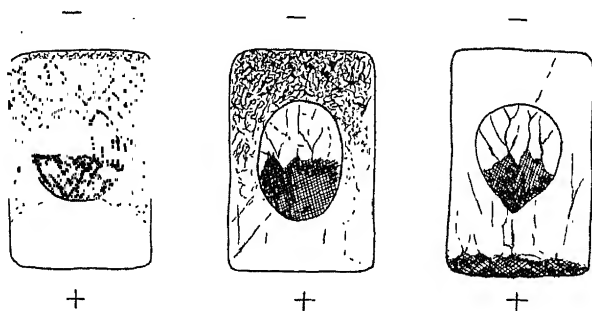


Fig. 6. Cataphoresis of protoplasm in cells of the onion root tip  
(after HARDY '13).

no way of knowing how much of the current actually passed through the cells themselves.

HARDY states that, "The results obtained were decisive and consistent". The protoplasm collected typically at the negative end of the cell, although sometimes it was condensed into an equatorial plate. This is shown clearly in A and B of Fig. 6, which is copied from HARDY's paper. However, after prolonged exposure to a strong field, the protoplasm was condensed at the positive end of the cell as shown in C of the figure. To quote HARDY further, "The appearances in the cell substances are such as would be due to the simple migration of colloid particles in a sol, that is to say of electrically charged particles distributed through a fluid. The sign of the particles being at first positive, they move towards the negative end of the cell." Here reversal of the charge occurs, and the particles move in the opposite direc-

tion\*. Concerning HARDY's observations on the migration of particles within the nucleus, we shall speak later.

HARDY's description indicates that the colloidal particles of protoplasm may reverse their charge after the passage of an electric current. It is not necessary to suppose, as he does, that the particles first move through the cell before the charge is reversed. In an earlier chapter (see p. 130), it was shown that in many types of protoplasm, electric currents cause coagulative changes. These coagulative changes may very well be associated with a loss of charge. If the original charge of the protoplasmic granules were positive, and this charge were lost following the passage of an electric current, it would seem likely that a negative charge would be assumed. In solutions near neutrality, most inanimate suspensions are charged negatively.

The observations of KÜHNE, VELTEN, and HARDY all indicate that in weak electric fields there is a cataphoretic migration of protoplasmic particles toward the cathode, and their results speak for the positive charge of the colloidal elements of the protoplasm. Similar results were apparently also obtained by ZEIDLER '25. HARDY's results are especially instructive in that they serve as a guide to the understanding of what might otherwise seem conflicting results. With too strong currents, currents that produce coagulation of the protoplasm, it is easy to understand how it is possible for migration to occur in the opposite direction.

Consider for example the results of MEIER '21. This worker sent electric currents through the roots of onion, lupine, pea and bean plants. The pea roots were studied most. Instead of placing the root in a watery solution, as HARDY did, MEIER attached two ends of the root to non-polarizable electrodes. The seedlings were held in position by glass forks in a moist chamber so that the current passed through the length of the root. An electric light current was used, the current actually passing through the root being measured by a milliammeter. In HARDY's experiments only a small fraction of the current passed through the root tissue. In

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\* It might be argued that the movement of the granules is due to the fact that they are carried by a current of water produced by electro-endosmosis. But this can scarcely be true, for according to HARDY the nucleus does not shift its position at all.

MEIER's experiments all the current went through the root. It is obvious that MEIER used very much more powerful currents than those used by HARDY. He found indeed that after about 30 seconds exposure to a current of 0.3 milliamperes, the root began to lose its normal color and became watery in appearance. After about two minutes, numerous fine droplets appeared over the surface about 3—6 mm. from the tip of the root. The root became very appreciably shorter. MEIER himself states that the effects he observed were produced by currents just strong enough to cause death. In MEIER's experiments, the protoplasm was certainly coagulated. His results are therefore comparable to those obtained by HARDY with strong currents for relatively long exposure. For currents which produce death, MEIER found a movement of protoplasmic particles toward the anode.

There can be no question but that the currents used by MEIER were too strong. Various other observers, from JÜRGENSEN in 1860 until the present, have also used too strong currents, so that their results give no information regarding the normal electric charge of the colloidal particles of living protoplasm. VERWORN '90 in studying the effect of electric currents on the plasmodia of the myxomycete *Aethalium septicum*, found that the particles of the protoplasm might migrate either to the anode or the cathode if they migrated at all. He states that he could find no regularity in their behavior. From his description of his experiments, he seemed to have used stronger currents than KÜHNE, for the electric current always caused a cessation of movement when it was first passed through the protoplasm. TAYLOR '25, in what is apparently a preliminary note, also reports observations on the cataphoresis of myxomycete protoplasm. He used micro-electrodes in a small hanging drop. TAYLOR states that the visible granules moved toward the anode, but it is possible that he did not make a clear distinction between protoplasmic granules and ingested material. For he says that following LISTER, (Ann. of Bot., vol. 2, p. 1), the granules may be filtered out with cotton. LISTER himself found that only some ingested spores were so filtered, but that the true granules passed through the cotton. TAYLOR also observed ultramicroscopic particles and noted that they moved either toward anode or cathode. Unfortunately the conditions of the experiment were not very satisfactory. Microelectrodes have a great resistance, and the

heat produced might injure the protoplasm. Moreover, in tiny droplets of fluid, electro-osmotic currents might be important. Finally, TAYLOR does not state how the passage of the electric current affected the normal streaming of the plasmodium, so that it is not possible to compare his observations with the previous observations of KÜHNE and VERWORN. MC CLENDON '10 figures a section of a hyacinth root through which a current of 0.5 milliamperes had been sent for 30 minutes. The protoplasm has moved toward the anode. MC CLENDON '14 states that both in animal and in plant cells there is a migration of "many substances of or in the protoplasm" toward the anode, but he does not state which cells he used in his experiments, or what strength currents. KÔKETSU '23 seems to have used too strong currents. He sent 50 volts through the hair cells of *Tradescantia* in air, and found a migration of cell contents toward the anode. In KÔKETSU's experiments, the current caused an immediate cessation of streaming, and evidently produced coagulation.

So far we have discussed only the cataphoresis of protoplasmic granules of plant cells. At the very end of the nineteenth century and at the beginning of the twentieth, many observers studied the effects of electric currents on protozoa. The physiological journals from 1896 to 1906 are full of papers which deal with this subject. Most of these papers are concerned primarily with the reactions of the animals and the behavior of the cilia, and there are unfortunately very few observations on the interior protoplasm. What there are, all favor the view that the colloidal particles of the protoplasm in the interior of protozoan cells are charged positively.

VERWORN '89, '90, '96a, describes the effects of electric currents on ameba protoplasm. During the passage of an electric current, the granules in the interior of the ameba all migrate toward the cathode. The figures in VERWORN's '96 paper are especially clear. As a matter of fact, the observation has been confirmed by various other workers. Whereas it is certain that the movement of the granules of the ameba protoplasm toward the cathode is consistent with the idea that they bear a positive charge, it must not be overlooked that this migration may very well depend on other factors. According to the leading theory of ameboid movement, the advancing pseudopodium of ameba has at its surface a region of lowered surface tension. It might

be thought that the electric current would cause a drop of surface tension at the cathodic side, and that the movement of the ameba would then correspond to the movement of a drop of mercury in an electric field, as studied by CHRISTIANSEN\*. However in order to assume such an effect of the electric current, we should have to suppose that the surface charge of the ameba cell was positive, and this is contrary to the evidence which we shall cite later,

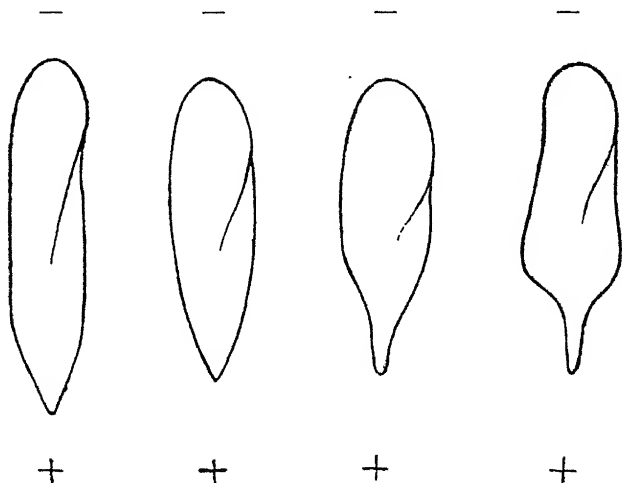


Fig. 7. Effect of an electric current on the shape of paramecium, after  
VERWORN '96

which indicates that the surface of living cells bears a negative charge (see p. 182).

The studies of paramecium seem to give clearer information. In the ameba, the movement of the granules under the influence of the electric current was not distinguishable from the movement of the granules as it occurs during the normal movement of the animal. One can not be certain therefore whether the migration of the granules toward the cathode depends primarily on electric forces, or whether it is not an end result due to the

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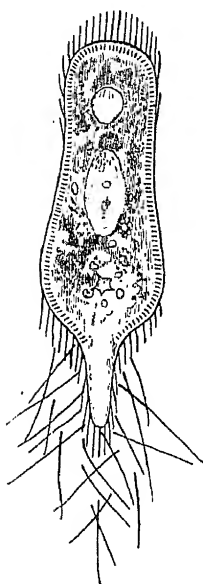
\* CHRISTIANSEN 1903, Ann. d. Physik, 4th ser., vol. 12, p. 1072.

movement of the entire animal. In the case of paramecium, there is no such difficulty. Before we examine the experimental evidence, let us inquire into the conditions of cataphoresis in the paramecium cell. Apparently this cell is full of granules. After long centrifugal treatment, there is no observable movement of granules, (see p. 81). This is probably an indication that there is no room for the granules to move, that they are too closely packed.

What then can happen when we subject such a cell almost solidly full of granules to an electric field? The granules are doubtless charged, and they would tend to move in one direction or the other. They would press against the wall on one side the cell, and if there were enough space between the granules, they would leave a small region at the opposite end of the cell totally free from granules. This is exactly what does happen in paramecium. LUDLOFF '95 sent electric currents through paramecia suspended in a gelatin gel to keep them motionless. When currents of about 1 milliampere were used, the granular endoplasm of the cell pushed toward the cathode. On the passage of an electric current, the cell bulges toward the cathode, and when the protoplasmic granules have left the side of the cell toward the anode, the wall in this region tends to collapse, thus resulting in the formation of a sharp point at the anode end of the cell. This is clearly shown in a series of diagrams taken from VERWORN '96a. These are reproduced in Fig. 7. One of LUDLOFF's figures is also shown in Fig. 8.

If the paramecium cell is full of granules, and if these are positively charged, there is hardly any other way in which they could respond to the passage of an electric current than that shown in the figures. The evidence therefore is good that the granules of the paramecium protoplasm are actually charged positively.

From our survey of the literature on cataphoresis in living cells, it seems not unfair to conclude that the weight of the evidence



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Fig. 8. Effect of an electric current on paramecium protoplasm after LUDLOFF '95



favors the assumption of a positive charge on the colloidal particles of the protoplasm. The observations of KÜHNE, VELTEN, HARDY on various types of plant tissue, together with the observations of LUDLOFF, VERWORN, and others on paramecium, all indicate a movement of granules toward the cathode. Probably cataphoretic studies are not possible for all types of protoplasm. It is likely that in many cases weak currents will cause coagulation, and once this has occurred, the normal charge of the protoplasmic particles is no longer in evidence.

As we shall see later, (p. 182), the charge on the exterior of a cell is normally negative. When cells are suspended in a solution of good conductance, normally most of the current does not enter the cells at all, and the migration of the cells in the electric field is determined entirely by the charge at the surface. But there is always some fraction of the current that passes through the cells, and if this is the case, the charges of the colloidal particles in the cell interior would be a factor in determining the speed and possibly also the direction of migration of the cells as a whole\*. We will assume for the present that cells generally bear a negative charge at the surface, at least in ordinary media. If this is true, and if it is likewise true that the colloidal particles in the interior of the protoplasm are positively charged, then as the size of the cell increases, the charges in the cell interior should become increasingly important. For, in the case of a sphere at least, the volume increases as the cube of the radius, the surface only as the square of the radius. This line of argument was used by HEILBRUNN '23, and he cites an experiment of R. S. LILLIE's to show that the size of a cell may have an influence on its cataphoretic migration. LILLIE '03 found that when frog blood cells were immersed in a sugar solution, the red blood cells and the smaller leucocytes, i. e. those about  $10\ \mu$  in diameter, almost always moved toward the anode. Leucocytes of medium size ( $15$  to  $20\ \mu$ ) were usually slightly negative, although occa-

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\* If the protoplasmic granules are positively charged and migrate toward the cathode, the suspension medium in the cell should migrate toward the anode. There would thus be an equal migration in both directions and the resultant force on the cell might be zero if it were not for the fact that the cell membrane is permeable to water and not to granules.

sionally indifferent or slightly positive. On the other hand, the more voluminous leucocytes (25 to 30  $\mu$ ) were almost always decidedly positive, moving definitely toward the cathode. LILLIE describes the appearance of cells in a given field moving in opposite directions. It is thus not probable that the movement of the large cells toward the cathode was due to their being carried in the stream of liquid which travels along the wall of the cataphoresis chamber.

In commenting on LILLIE's experiment, it is to be noted first that the cells which migrated in opposite directions were of the same type and from the same animal. It would hardly be profitable to compare the speed of migration of cells of different animals or of totally different types of cells from the same animal. Secondly in LILLIE's experiments the cells were immersed in a sugar solution. This would provide an outer medium of low conductivity, and as a result a relatively greater fraction of the current would pass through the interior of the cells. This is the very condition which would favor an influence of the interior charges of the protoplasm on the direction and speed of migration of the cells as a whole.

BERNARDI '26 diluted the blood of the mollusc *Phallusia* with sugar solution, one part blood to five parts sugar solution. Under these conditions, he found that the smaller cells migrated to the anode, whereas the larger yellow cells migrated to the cathode. In his description of his methods, BERNARDI states that he was careful to avoid deception from the movement of the film of fluid next to the glass.

When the cataphoretic migration of cells is studied in blood serum or in salt solutions, one would not expect to find very great differences between the rate of migration of the small and the large cells. That such differences in rate of migration actually do appear is at least indicated by the exact quantitative determinations of FREUNDLICH and ABRAMSON '27 on the cataphoretic rate of blood cells of the horse suspended in serum. From the data of these authors, the average speed of migration of various types of white blood cells has been calculated, and a comparison may be made between the speed of migration of small lymphocytes, large lymphocytes, and the presumably still larger leucocytes. In the following table, the figures represent the migration rate of the cells in microns per second for an electric field of one volt

per centimeter. FREUNDLICH and ABRAMSON separated their observations into two groups, those made one to six hours after the blood was obtained, and those made after 30 hours. In the table, these two sets of observations are also kept separate.

Cataphoretic migration rate of white blood cells of the horse  
in microns per second for one volt per cm.  
(FREUNDLICH and ABRAMSON '27)

	Blood 1—6 hours old	Blood 30 hours old
Small lymphocytes. . .	0.670	0.588
Large lymphocytes. . .	0.550	0.558
Leucocytes . . . . .	0.514	0.500

In view of the small number of observations reported by FREUNDLICH and ABRAMSON, these figures may not be statistically significant. It is interesting to note that the red blood cells, which have a volume still less than that of the small lymphocytes, were found by FREUNDLICH and ABRAMSON to have a greater speed, (1.01 microns per cm. for a field of one volt per cm.). But the comparison between different types of cells is probably not justifiable.

It might be thought that the slower rate of migration of larger cells in an electric field was due to the greater resistance offered by the medium to the passage of the cells. As a matter of fact, both from the standpoint of theory, and from the results of actual experiment, (cf. FREUNDLICH and ABRAMSON), the speed of migration of a particle in an electric field is independent of its size.

The experiments of LILLIE '03, BERNARDI '26, and FREUNDLICH and ABRAMSON '27 all indicate that with increasing size of a cell, it tends to move less rapidly toward the anode and may even move in the opposite direction. So far the evidence on this point is rather meager, but what there is favors the view that the interior of the cell contains particles charged positively.

Finally, there is a bit of indirect evidence, which although it is not as important as the evidence we have already considered, may be worth mentioning. KOEPPE '97 advanced the view that the red blood cells were permeable to anions, but not to cations. Following KOEPPE's paper, there has been an abundance of

evidence in support of this view, (for literature see HÖBER '26, pages 467 to 474). In general, membranes that retard the passage of anions as compared to cations are negatively charged, those that retard the passage of cations as compared to anions are positively charged, (see for example BETHE and TOROPOFF\*. The evidence with regard to the relative permeability of red blood cells to cations and anions indicates that the osmotic membrane of these cells is composed of particles charged positively\*\*, so that inside of the negatively charged surface there is apparently a region composed of positively charged particles. This of course does not prove that the colloids in the interior of the cell are likewise charged positively.

Summing up, we find the following evidence in favor of a positive charge on the colloids in the interior of living protoplasm:

1. The excess of bivalent cations in living cells.
2. The fact that bivalent and trivalent cations favor a liquefaction of the protoplasmic suspension, whereas a decrease in the proportion of bivalent cations normally present results in coagulation.
3. The cataphoresis experiments of KÜHNLE, VELTEN, and especially those of HARDY. All of these experiments indicate a migration of the protoplasmic granules of plant cells toward the cathode.
4. The movement of the granules in the interior of the paramecium cell toward the cathode.
5. The fact that when the cataphoresis of large and small cells of the same type is compared, the larger cells show a greater tendency to move toward the cathode.

It is of course quite possible that different types of protoplasm differ in the sign of the charge on their colloidal particles. So far there is no evidence to indicate that this is the case. All the types of protoplasm that have been studied seem to behave in the same manner.

In our discussion up to this point, we have considered only the charge on the colloidal particles or granules of the cytoplasm.

\* BETHE and TOROPOFF 1915, *Zeitschr. f. physik. Chem.*, vol. 89, p. 597.

\*\* This does not agree with the opinion of HEILBRUNN '23, '25a regarding the charge on the colloidal particles of the outer membrane of the sea-urchin egg, but this opinion was not based on very certain evidence.

There is good evidence that the colloidal particles of the nucleus are charged with an opposite sign from those of the cytoplasm, that is to say, that they are charged negatively.

The experiments of HARDY '13 previously referred to, very clearly demonstrate a migration of the materials within the nucleus toward the anode following the passage of an electric current. This is shown in all three of the sketches in Fig. 6. Apparently the colloids of the nucleus do not reverse their charge as do those of the cytoplasm, but remain negatively charged at all times. HARDY reached his conclusions regarding the negative charge of the colloidal particles of the nucleus apparently quite independently of the earlier work of PENTIMALLI and MC CLENDON.

PENTIMALLI '09 passed currents of from 0.0186 milliamperes to 0.0492 milliamperes through hyacinth root tips for 20 to 45 minutes. He used non-polarizable electrodes, and in different series of experiments, the current was sent in various directions through the root. PENTIMALLI was able to demonstrate a movement of the chromosomes of dividing cells toward the anode. However the chromatin of resting nuclei showed little or no movement. MC CLENDON '10 repeated PENTIMALLI's experiments, using approximately the same strength of current for 30 minutes. Both in the onion root and in the root of the hyacinth, MC CLENDON was able to demonstrate a movement of chromatin toward the anode. But MC CLENDON was able to show not only a movement of the chromatin in dividing cells, but also a movement of the chromatin in resting nuclei. His figures leave no question of doubt. MC CLENDON states also that the chromatin of the cells of the newt *Diemyctilus* moves toward the anode, and that nucleoli of frogs' eggs travel in the same direction. He gives no details of these experiments with animal cells. In a second paper, PENTIMALLI '12 repeated his earlier experiments on the hyacinth root, using stronger currents. In this paper his figures show very clearly a migration toward the anode of chromatin both in resting and in dividing cells. ZEIDLER '25 has also described a migration of chromatin toward the anode.

PENTIMALLI, MC CLENDON, HARDY and ZEIDLER, agree in ascribing a negative charge to the colloidal particles of the nucleus, and it seems certain that in the cells studied by them such a charge is actually present. The experimental procedure used by

the above-mentioned authors is simple, and it is rather surprising that more experiments of the same type have not been performed. Surely the electric charge on the chromatin is an important subject for investigation. By sending an electric current through cells for varying lengths of time, some idea of the magnitude of the charge might be obtained, although in order to determine the charge exactly, a knowledge of the relations of the current within the nucleus would be necessary. Other types of cells could also be studied, or the cells could be treated in one way or another to see what effect such treatment might have on the electric charge.

As far as the evidence goes, the colloidal particles of the interior cytoplasm bear a positive charge, those of the nucleus a negative charge. We shall now consider the charge on the surface of the cell.

The charge on the colloidal particles of the cell interior is determined largely by the ions present inside the cell. Concerning these ions we have little definite information, except that apparently there is an excess of inorganic cations. The charge on the outer surface of the cell would be determined largely by the ions present in the outer medium. Concerning these ions we have complete information, or at any rate we may have.

The outside membrane of the cell is probably protein. Most proteins in solutions near the neutral point, and in the absence of cations of high valence, are charged negatively. It is not surprising, therefore, to find that in cataphoresis experiments generally, living cells have been observed to migrate toward the anode. This is particularly true for small cells such as red blood cells and bacteria, for in such cells the charges of the interior play less of a rôle. The first description of cataphoresis of living cells is that of JÜRGENSEN '60. He found that blood cells migrated toward the anode. Since JÜRGENSEN, there have been very many papers published on the cataphoresis of living cells, and it would scarcely be possible to refer to all of them. The more recent workers, especially following the publication of convenient directions by MICHAELIS '21, have usually been careful to guard against the error due to the movement of the fluid in the cataphoresis chamber. The following papers may be consulted, some of them contain references to older literature: v. SZENT-GYÖRGYI '20, PÜTTER '21, NETTER '25, TOMITA '26, BERNARDI '26, WINSLOW and UPTON '26, SCHRÖDER '26.

Just as the charge of an inanimate particle is influenced by the ions of the surrounding medium, so too the charge on the surface of living cells is altered when various ions are added to the fluid surrounding the cells. In the presence of a sufficient amount of bivalent cations, the charge on living cells may be changed from negative to positive, (see for example BERNARDI '26). Trivalent ions are especially effective in producing a change in direction of electric migration of living cells, (see for example KOZAWA '14).

Our discussion has shown that the granules in the cytoplasm of living cells bear a positive charge, that the materials of the nucleus have a negative charge, and that the surface of the cell is usually charged negatively. There is also some indirect evidence that the colloidal particles of the osmotic membranes of cells, blood cells at least, have a positive charge. It should be clearly understood that the charges on colloidal particles, living or non-living, are not free charges. They can not be made to travel along a wire. For each positive charge on a colloidal particle or micella, there is a negative charge in the dispersion medium immediately adjacent. The potentials that we have been considering are moreover all electrokinetic potentials, or  $\zeta$  potentials. It is this type of potential that is measured in cataphoresis experiments\*.

In recent years, various authors have attempted to determine the difference in thermodynamic potential or  $\epsilon$  potential between the inside of living cells and the outside, (see ETTISCH and PÉTERFI '25a, b; PÉTERFI, see KELLER '25, TAYLOR and WHITAKER '26, OSTERHOUT, DAMON and JACQUES '27, GICKLHORN and UMRATH '28). Differences in the thermodynamic electric potential between the inside of a cell and the outside may be due to diffusion potentials, to potentials resulting from the differential permeability of the cell membrane to anions and cations, and possibly to other factors. In some of the experiments cited above, there is also the possibility that an injury potential may have played a rôle. The results that have been obtained, although very interesting, have no very direct bearing on the colloid chemistry of protoplasm.

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\* For a discussion of the difference between electrokinetic potential and thermodynamic potential, see recent books on colloid chemistry, especially ETTISCH 1927, *Die physikalische Chemie der kolloiden Systeme*. In Bethe's *Handbuch der normalen und pathologischen Physiologie* vol. 1, p. 91, Berlin 1927.

## CHAPTER XI

# THE ACTION OF ACIDS AND ALKALIES

The study of the action of acid on protoplasm is beset with unusual difficulties. Indeed it must be confessed at the outset that for the most part these difficulties have not been overcome, and that our present knowledge regarding the action of acid on protoplasm is rather unsatisfactory.

The older physiologists who experimented with acids compared solutions of similar normalities. In recent years it has been clearly recognized that two solutions of the same normal concentration may differ widely in acidity, that is to say, in hydrogen ion concentration. One may be thousands of times as acid as the other. With the development of simple and easy methods of measuring hydrogen ion concentration, and with the recognition of the importance of slight differences in such concentration on the life of plant and animal cells, biologists have now generally learned to express acidity in terms of hydrogen ion concentration. Every beginner in physiology is now taught to measure the  $\text{cH}$  or  $\text{pH}$  of the solutions he uses in his experiments.

Unfortunately the assumption is often made that two solutions of the same hydrogen ion concentration are strictly comparable in so far as the effect of the acid ion on protoplasm is concerned. As a matter of fact, in two solutions of the same  $\text{pH}$ , the amount of acid which enters the cell in a given time may be vastly different. Consider for example a solution of a strong mineral acid and a weak organic acid. At the same hydrogen ion concentration, the organic acid has a concentration thousands of times as great as that of the mineral acid. Because of this difference in concentration, its diffusion rate is enormously greater and very much more acid enters the cell. If the cell were a sack containing pure water, this difference in diffusion rate would not be important, for because of the weakness of the organic acid, proportionally more acid would have to enter to



produce the same degree of acidity. But the cell contains various substances which combine with acids. These probably combine with both weak acid and strong acid in the same stoichiometric proportion. The normality of the entering strong acid is much lower than that of the weak acid; the strong acid is thus more rapidly used up than the weak acid. It is thus easy to see that a weak acid may produce more acidity within the cell than a strong acid of the same hydrogen ion concentration.

As a matter of fact, it has often been found that organic acids are much more toxic for living cells than inorganic acids of the same hydrogen ion concentration, (see for example BRENNER '18, and literature cited by him). This greater toxicity of weak acids has generally been interpreted as being due to a difference in the permeability of the plasma membrane to different types of acids. Such a difference in permeability doubtless plays a part, but certainly the difference in diffusion rate is also an important factor.

When cells are placed in buffered solutions of different hydrogen ion concentrations, it is obvious that the pH of the interior of the cell is not the same as the pH of the solution surrounding the cell. In order that the two be the same, we would have to assume that buffer and acid penetrated the cell at the same rate of speed, and that moreover the cell contained no buffers of its own. It is easily possible to conceive of cases in which increase in the hydrogen ion concentration of the outer medium might be accompanied by a decrease in the hydrogen ion concentration of the cell interior, or vice versa. Such a case has been noted experimentally by JACOBS '20. In this instance, carbonic acid was present in high concentration in the outer medium, but the reaction of this outer medium remained alkaline because of the presence of carbonates. Being a weak acid, the carbonic acid had little effect on the external pH, but because of its high concentration it diffused rapidly into the cell and there produced acidity.

In interpreting the results of experiments with acids, we must therefore remember that the hydrogen ion concentration of the outer medium is not an accurate measure of the changes in acidity that occur in the cell interior. However, by a suitable choice of solutions, we can at least be reasonably certain that an increase in the acidity of the outer medium is reflected by a

parallel, even though not by a proportionate increase in the acidity of the interior. Not absolutely certain perhaps, for protoplasm is constantly producing acid and perhaps also alkali, and it is quite possible that with increase in alkalinity of the external medium, there is an increase in the cellular production of acid. Indeed it is sometimes stated that alkalies do actually produce an increase in the rate of metabolism, that is to say an increase in the amount of carbonic acid manufactured by the cell.\*

In view of all these uncertainties, we must be very careful not to draw too far-reaching conclusions from the experimental evidence that we now have at our disposal.

The action of acid and alkali on protoplasm has been studied in various ways. One or two authors have investigated viscosity changes, there have been a few observations on the effect of acid on protoplasmic streaming, and finally several authors have described the morphological changes which follow acid treatment both in brightfield and darkfield.

JACOBS '22 studied the effect of carbon dioxide on the protoplasmic viscosity of *Paramecium* and *Colpidium*. In these Protozoa the granules do not move very readily under the influence of centrifugal force. JACOBS overcame this difficulty by feeding the organisms India ink. Vacuoles containing India ink appeared within the protoplasm, and these moved very readily when the animals were centrifuged. JACOBS bubbled carbon dioxide through the fluid in which the organisms were swimming. He found that short exposure to this carbon dioxide produced a marked liquefaction in the protoplasm of both *Paramecium* and *Colpidium*. Longer exposure caused an increase in viscosity. In *Paramecium*, it was possible to show that in this stage of increased viscosity, the viscosity was greater than at the beginning of the experiment. In *Colpidium*, the viscosity was at least as great, but it could not be shown to be greater.

JACOBS also studied the action of carbon dioxide on the protoplasmic viscosity of sea-urchin eggs. When *Arbacia* eggs were exposed for five minutes to sea-water saturated with carbon-dioxide, centrifuge tests showed coagulation. The pH of the sea-water saturated with carbon dioxide was 5.5. When the sea-water was made acid by adding strong acid, and the carbon

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\* Compare for example LOEB and WASTENEYS '13.

dioxide was removed by shaking, at a pH of 5.5 no coagulation of the protoplasm was produced. Apparently, therefore, at the same pH, carbon dioxide is more effective in producing coagulation than is strong acid. From what has been said previously, this is readily understandable. At a pH of 5.5, the concentration of carbon dioxide present is great, vastly greater than the concentration of mineral acid which would be present at this pH. The carbon dioxide thus diffuses much more rapidly into the cell.

According to JACOBS, the coagulative effect of carbon dioxide on *Arbacia* protoplasm is reversible. After a five minute exposure, the protoplasm returns to its original fluid state.

JACOBS also performed some experiments with *Spirogyra*, centrifuging the filaments after exposure to carbon-dioxide saturated water. Here too, short exposure caused liquefaction, whereas longer exposure caused coagulation. With one set of material this coagulation was apparently reversible, but in other material it was associated with the death of the cells.

Summing up these results, it is seen that carbon dioxide produced both a liquefaction and a coagulation in the protoplasm of Protozoa and *Spirogyra*. In the *Arbacia* egg, JACOBS records only coagulation. The coagulation is generally reversible, although in one set of experiments with *Spirogyra* this was not found to be the case.

That carbon dioxide may cause a reversible increase in protoplasmic viscosity is also indicated by the older experiments of KÜHNE '64 and LOPRIORE '95. The latter treated stamen hairs of *Tradescantia* with mixtures of carbon dioxide gas and air. He found that 80 % carbon dioxide caused cessation of protoplasmic streaming after 3 to 4 minutes. When the stamen hairs were returned to air, the protoplasm began to stream again.

It is of course an open question whether or not the effects produced by carbon dioxide can be attributed to the hydrogen ion or to the undissociated gas present in solution. JACOBS himself inclines to the view that the action of carbon dioxide is specific, and he cites experiments in which mineral acids at the same pH do not produce as powerful an effect as carbon dioxide. This is however readily understandable on the basis of our previous discussion, for at the same pH, carbon dioxide is vastly more concentrated than the mineral acid. On the other hand, there is good reason to believe that carbon dioxide

in solution may produce effects quite apart from its acidity. A solution of carbon dioxide in water has a lower surface tension than the pure solvent, and might very well act as a fat solvent. In this connection, it is interesting to note that carbon dioxide is often used as an anesthetic in physiological experiments, and most anesthetics are known to dissolve fats. Fat solvents typically produce a decrease in protoplasmic viscosity, (see chap. 12), and perhaps the liquefaction caused by carbon dioxide is of this nature.

Before going further, it should be pointed out that the addition of any acid may result in the liberation of carbon dioxide within the cell. Very probably protoplasm contains carbonates, and these would be broken down in the presence of most acids. Thus when we treat cells with acid, we can never be sure that the effect is not due to carbon dioxide.

The most extensive experiments on the effect of acid on protoplasmic viscosity are those of SAKAMURA and LOO '25. These authors centrifuged *Spirogyra* filaments after exposure to solutions of varying pH. The exposure time in all of their experiments was 5 minutes.

In order to obtain solutions of different pH, SAKAMURA and LOO used solutions of the two following types:

#### Type A

n/100 NaOH . . . . . 2 cc.  
 n/100  $\text{H}_3\text{PO}_4$  . . . . . various amounts (0.6—2.2 cc.)  
 Diluted with water to 20 cc.

#### Type B

n/50  $\text{Na}_2\text{HPO}_4$  . . . . . 1 cc.  
 n/500 HCl\* . . . . . various amounts (0.2—10.55 cc.)  
 Diluted with water to 20 cc.

In all, SAKAMURA and LOO performed eleven experiments or series of experiments. The first six experiments were performed during the last week in February and on the 4<sup>th</sup> and 8<sup>th</sup> of March. In these experiments, a solution of Type A was used. The other five experiments were performed from the 7<sup>th</sup> to the 11<sup>th</sup> of

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\* In the original paper the acid is given as HCl, but this is doubtless a misprint.

July, and the filaments were immersed in a solution of Type B. The pH varied from 3.5 to 8.2. In measuring the viscosity, SAKAMURA and LOO noted the percent of cells which showed movement of chromatophores after treatment with centrifugal force. They distinguished four different degrees of chromatophore displacement, from no displacement at all to a very complete displacement of chromatophores, and they counted the percents of cells which showed each of these four degrees of displacement.

From their data, SAKAMURA and LOO conclude that as the pH is changed from 3.5 to 8.2, the protoplasmic viscosity goes through three successive stages of increase and decrease. That is to say, if one were to plot a curve, the abscissae of which were pH values and the ordinates viscosity values, one should obtain a three-peaked curve, i. e. one with three maxima. SAKAMURA and LOO actually figure such a curve, but they assign no definite values either for pH or for viscosity, and the curve as it stands merely represents the general conception of the authors. The points of high viscosity in the curve, SAKAMURA and LOO regard as isoelectric points, but in a postscript they state that if protoplasm is a sol instead of a gel, the points of low viscosity represent the isoelectric points. Although these points of maximum and minimum viscosity are not located on the curve that SAKAMURA and LOO draw, they are definitely fixed in the following table, which is copied from the original paper. It will be noted that the position of the points varies with the seasons, and SAKAMURA and LOO state also that it varies with other external conditions.

Table I. Points of maximum and minimum viscosity in *Spirogyra* protoplasm according to SAKAMURA and LOO

For plants in the winter and spring.

Minimum viscosity. . . .	<4.7	6.0	6.8	
Maximum viscosity. . . .	4.9	6.4	7.5	

For plants in the summer.

Minimum viscosity. . . .	<4.7	5.8	7.2	
Maximum viscosity. . . .	5.3	6.4	7.6	

If it is true that protoplasm goes through successive stages of high and low viscosity as the pH is varied, this is extremely interesting. In view of the fact that SAKAMURA and LOO's work has been generally accepted and frequently quoted, it may be worth while to inquire into the justification for their statements.

The appended tables give all of SAKAMURA and Loo's data for least and greatest degree of displacement of chromatophores. The figures in Table II. are in percents of cells which show no displacement of chromatophores, those in Table III. the percents of cells which show very strong displacement. It is hardly necessary to include data concerning intermediate degrees of displacement, for these appear to be less certain. Each row of figures in the tables is a single experiment. The first column gives the number of minutes the cells were centrifuged, and the other columns the percentage of chromatophore displacement or non-displacement at different hydrogen ion concentration. In all but the 4<sup>th</sup> experiment, the centrifuge turned 2000 times per minute. In experiment IV it turned 1500 times. It is to be remembered that the first six experiments were performed in the winter and spring, the other five experiments in the summer.

SAKAMURA and Loo's observations, when collected together in the form in which they are presented in Tables II and III. scarcely carry conviction. The data are not consistent. Let us compare for example the third experiment in which the filaments were centrifuged for 2 minutes with the fifth experiment in which the filaments were centrifuged at the same speed for 3 minutes. The shorter centrifugal treatment perhaps accounts for the fact that the percentage of cells which showed no displacement of chromatophores at pH 6 is 4 times as great for the third experiment as it is for the fifth. But how explain that at pH 4.0 and at pH 4.3 the percentage of displacement is two or three times as great for the fifth experiment as for the third? Examples of this sort can be multiplied many times. The reader may easily find them for himself.

Let us consider now the evidence for SAKAMURA and Loo's points of high and low viscosity. To take one example, for winter plants there is thought to be a point of maximum viscosity at pH 7.5. In all there are only two measurements at this pH. In the first experiment, 40 % of the cells showed no displacement at a pH of 7.5. This percent is a little over the average for the series, although not much. On the other hand, in the only other experiment, the second, 20 % of the cells showed no displacement at pH 7.5, and this value instead of being high, is next to the lowest one of the whole series of ten determinations. Again, for summer



plants, pH 5.3 is said to be a point at which the viscosity is at a maximum. What is the evidence? There are no measurements at pH 5.3. If one averages the ten determinations at pH 5.2 and pH 5.4, one obtains a value of 51.8 %. At pH 5.6, an average of 4 determinations gives a value of 52 %, and at pH 5.8 an average of 4 determinations, 51 %. How then a maximum of viscosity? Finally for the summer plants, SAKAMURA and LOO state that below pH 4.7 the protoplasm is highly fluid. But in the data that they offer, they show no record of tests at any pH below 4.7.

It is indeed doubtful if a single one of SAKAMURA and LOO's points of high and low viscosity has any justification on the basis of the experimental data that they have published. All that the tables show is that the material they used in their experiments was highly variable.

ROBBINS '26 exposed *Elodea* leaves to various sodium phosphate solutions in which the pH varied from 4.8 to 7.2. Following a four hour exposure to these solutions, the leaves were centrifuged for 5 minutes at speeds of 650, 1000 and 2000 revolutions per minute. "The chloroplasts were thrown to one end of the cell in those centrifuged at 2000 revolutions per minute. Those centrifuged at 650 revolutions per minute were almost unaffected and those at 1000 revolutions were not affected much. No difference between the leaves from the acid and alkaline end of the series could be detected." ROBBINS rather expected to find such differences, for as will be shown later, he found significant differences in streaming rate. Perhaps differences in viscosity might have been noted if tests had been made at speeds intermediate between 1000 and 2000 revolutions per minute. But it seems certain from ROBBINS' experiments, that there are no very marked viscosity differences.

JACOBS, SAKAMURA and LOO, and ROBBINS made use of the centrifuge method in their viscosity determinations. PRÁT employed WEBER's plasmolysis-form method in studying the effects of acid and alkali on cells of the onion. He added acid or alkali to sea-water\*, and he then used the resultant solutions as plasmolytic

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\* PRÁT does not state whether or not he removed the  $\text{CO}_2$  from the acidified sea-water. If he did not, this might have a bearing on the interpretation of his results.



agents for the onion cells. He states that, in general, acid solutions are more apt to produce a perfect convex plasmolysis. In neutral or alkaline solutions, the plasmolysis is imperfect or concave. Only in relatively high concentrations of acids does irregular or concave plasmolysis occur. Hydrochloric and sulphuric acids produce a concave plasmolysis at a pH of 2. Acetic acid produces the same result at more dilute hydrogen ion concentrations (pH 3 or 4 or 5). This is readily understandable on the basis of the discussion at the beginning of the chapter.

PRÁT's results indicate that with an increase in hydrogen ion concentration, there is a progressive decrease in viscosity or a liquefaction of the protoplasm, until finally when the concentration of hydrogen ions becomes too great, coagulation sets in. In addition to these observations on the plasmolysis form, PRÁT also made some morphological observations which will be referred to later.

In this connection it is well worth while to consider the effect of acid on the rate of protoplasmic streaming in plant cells, in spite of the fact that these results are always capable of a double interpretation, (see p. 51). KLEMM '95 studied the effect of rather high concentrations of various acids on protoplasmic streaming in several different sorts of plant material. He found that the streaming first became irregular, and finally stopped. NOTHMANN-ZUCKERKANDL '12 also found that various acids caused a stoppage of protoplasmic streaming. LAKON '14 observed that in *Elodea* cells 0.005 % sulphuric acid initiated streaming in 1—2 minutes. In this short time, it is hardly probable that the dilute sulphuric acid penetrated the cells, and it seems likely that the action of the acid was primarily on the cell surface, where it might very well have produced some change which would affect the driving force responsible for the streaming.

ROBBINS '26 studied the effect of solutions of different hydrogen ion concentrations on the rate of streaming in *Elodea* leaves. Thirteen sodium phosphate buffer solutions were used, and in these the pH varied from 4.8 to 7.2. The leaves were kept in the solutions for 5—6 days, following which rates of streaming were determined by noting the time required for a plastid to pass a measured distance. These times are given in the following table. Each time recorded is the average of 25 determinations.

Table IV. Inverse speed of streaming in *Elodea* cells at different hydrogen ion concentration (ROBBINS '26)

pH	4.8	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2
Inverse speed	3.9	4.3	4.6	4.4	3.9	4.5	4.8	4.2	5.3	5.7	5.1	6.0	No streaming

From these observations, ROBBINS concludes that there is a slower rate of streaming in solutions of pH 6.4 to 7.2. It will be remembered that ROBBINS found no differences in viscosity between the cells in the alkaline and those in the acid solutions. It should be noted that in his viscosity tests, ROBBINS exposed the leaves for 4 hours instead of for 5 to 6 days. The evidence is not very conclusive, but as far as it goes it indicates that the differences in rate of streaming do not depend on differences in protoplasmic viscosity.

STRUGGER '26 studied the effect of solutions of differing hydrogen ion concentration on the rate of protoplasmic streaming in the root hairs of *Hordeum vulgare* (barley). He found the rate of streaming to increase from pH 6.8 to pH 6.4, then to decrease markedly to a minimum value at about pH 6.15. At this point, the protoplasm stops streaming. Then, as the pH is lowered from 6.15 to 5.85, the protoplasm flows ever more rapidly, reaching a maximum speed at the latter point. With further increase in hydrogen ion concentration, the speed of streaming rapidly falls off until it stops completely at pH 5.65. This is the lethal concentration. Such a succession of maxima and minima are very interesting, suggesting as they do possible isoelectric points in the protoplasm. We shall therefore examine the experimental evidence closely.

The solutions used by STRUGGER contained sodium acetate and acetic acid in differing proportions. STRUGGER does not clearly state the concentrations of the solutions he used, but apparently at the higher concentrations of hydrogen ion, the acetic acid reached a concentration of 0.0032 normal. Solutions of this concentration might very conceivably have an effect on the surface tension of the cell, for acetic acid in aqueous solution does have a markedly lower surface tension than the pure solvent. STRUGGER usually began his measurements very soon after the immersion of the root hairs in the solutions. In three of the fifteen experiments he cites, he began measuring after 2 minutes, in

another experiment after only 1 minute. Ten determinations were made in each experiment, and these required a time of 3 to 15 minutes. It is interesting to note that the measurements made at the end of this period do not differ appreciably from those made at the beginning. When the root hairs are immersed in the solutions, apparently an equilibrium is reached within 2 or 3 minutes, or possibly only 1 minute. This could hardly be the case if the acid produced its effect only after diffusing into the protoplasm. There is evidence, therefore, that the results of STRUGGER are, to some extent at least, due to a surface action of the solutions he used. This is borne out by the fact that STRUGGER clearly states that the volume of the protoplasm increases in certain of his solutions. Such an increase in volume might easily be the result of a lowering of surface tension of the protoplasm. The volume increase indicated in the diagrams and photographs of STRUGGER is a very large one, and would certainly result in a decided decrease in the protoplasmic viscosity, quite apart from any effect of the H ion.

Rather a clear indication that the results of STRUGGER with acetic acid are due in some measure to a surface tension effect, is the fact that he finds solutions of chloroform give the same morphological picture as the acid solutions. Both in the presence of chloroform and in the presence of acetic acid, the protoplasm shows increase of volume with occasional bursting of the cell wall ("Plasmotypse"), formation of new vacuoles, appearance of new granules, and also what STRUGGER calls regeneration phenomena.

It seems clear that in STRUGGER's experiments, surface tension lowering, (and perhaps also a fat solvent action of the acetic acid) play a large rôle. From the morphological side, STRUGGER describes both an increase in the volume of the protoplasm and the formation of new granules within the protoplasm. The increase in volume would of course tend to produce a lowering of protoplasmic viscosity, (for actual data, see p. 212), whereas the formation of new granules would certainly increase the viscosity. When the root hairs are subjected to various concentrations of acetic acid there are thus two factors operative, one of which tends to decrease the viscosity and the other of which tends to increase it. As one or the other of these two factors gains the ascendancy, it is easy to see that the vis-

cosity would go up or down, and we might have any number of maxima or minima if we plotted viscosity or speed of streaming against hydrogen ion concentration. This is almost certainly a partial if not a complete explanation of the peculiar results obtained by STRUGGER. It should be noted that this explanation does not depend on the assumption that surface tension lowering is responsible for the phenomena, but merely on the morphological observations made by STRUGGER.

In a paper which has just been published, STRUGGER '28 again studies the effect of various hydrogen ion concentrations on barley root tips. The material is thus the same, but in the newer experiments the hydrogen ions are due to phosphoric acid. There is thus not the same difficulty in regard to surface tension, but unfortunately in STRUGGER's new experiments he introduces another complicating factor. Plant roots are very sensitive to osmotic conditions. The ordinary nutrient solutions in which they can live are very dilute. The solutions which STRUGGER '28 uses are apparently too concentrated, and moreover they do not have the same osmotic strength. Between a pH of 6.8 and 7.5, there is a variation from approximately  $m/15$  to  $m/25$  solutions. With these solutions, STRUGGER reports various injurious effects at hydrogen ion concentrations just under neutral. This is quite contrary to the older work of KAHLENBERG and TRUE '96 and HEALD '96. These authors found that roots of *Lupinus* and *Curcubita* could live and continue to grow for at least two days in  $n/6400$  solutions of hydrochloric, nitric, sulphuric, and other mineral acids, whereas *Zea* roots could even withstand  $n/3200$  solutions. An  $n/6400$  solution has a pH of about 3.8. KAHLENBERG and TRUE exposed the roots for two days. For shorter exposures, BRENNER '18 found that various plant tissues showed a tolerance for higher concentrations of acid. MEVIUS '27 grew several types of plant roots at a pH of 3.3. ADDOMS '23 found no evidence of injury in roots of wheat seedlings grown at a pH of 3.68 for a week, (see below).

In addition to STRUGGER, various other authors have described visible changes in protoplasm following treatment with acids and alkalis. All observers agree that acids, when present in sufficient concentration, cause the appearance of new granules within the cell. KLEMM '95 describes "körnige Ausscheidungen", that is to say the precipitation out of granules, following treat-

ment with nitric, sulphuric, hydrochloric, phosphoric, chromic acids, as well as by citric, oxalic, acetic, and other organic acids. KLEMM used a concentration of 0.05 % nitric acid, and the other acids were used in about the same percentage strength. No statement is made regarding normality. The plant material used was of various sorts, root hairs of *Trianea* and *Mormodica*, leaf cells of *Vallisneria*, -epidermis cells of *Tradescantia*, etc. According to KLEMM, not only may acid cause the appearance of new granules, it may also cause the formation of many small vacuoles within the protoplasm. PRÁT '26 states that granules increase in number in onion cells following acid treatment. On the other hand, in the presence of alkalies there is a tendency for the granules to disappear.

A clear description of the formation of granules as a result of acid treatment is given by LEWIS '23. She studied cells in tissue culture. In these cells the cytoplasm is normally free from granules. LEWIS studied both cytoplasm and nucleus, and she states on the basis of morphological evidence, that coagulation of the nucleus occurs at a pH of 4.6 in all of the following acids: hydrochloric, sulphuric, nitric, acetic, lactic; citric, oxalic, and picric. That all of these inorganic and organic acids should produce an effect at the same pH is rather surprising, for the normal concentrations of the acids and their rates of diffusion into the cell must be markedly different, (compare discussion on p. 184). LEWIS claims that the coagulative effect of acids is reversible. That acid coagulation of protoplasm may be reversible is also indicated by the observations of VAN HERWERDEN '25. She studied human leucocytes in saliva. Normally these leucocytes show both ameboid movement and Brownian movement of granules in their interior. Both ameboid movement and Brownian movement ceased when the cells were placed in 0.1 % acetic acid (in 0.9 % sodium chloride). On washing with 0.9 % sodium chloride, the Brownian movement began again, also the ameboid movement.

LEWIS studied not only the action of acid, but the action of alkalies as well. She used solutions of ammonium hydroxide, ammonium carbonate, lithium carbonate, and sodium carbonate, and found that at a pH of 8.6 to 9 there was apparently an increase in the fluidity of the protoplasm. At any rate the cells tended to round up in these solutions. This is of course not very good

evidence of greater fluidity, as many factors may be involved in such a change in shape. The effect of the alkalis was reversible.

CZAPEK '23 states that BORESCH in his laboratory treated exuded protoplasm of *Vaucheria* with dilute alkali. Following this treatment, there was an appearance of droplets in Brownian movement. CZAPEK's statement is too brief to be very helpful. The appearance of droplets would indicate a viscosity increase provided that they were new formations. The fact that they were in Brownian movement might indicate a viscosity decrease, if there were no Brownian movement before treatment. Unfortunately CZAPEK's words are ambiguous.

In plant cells, alkalis cause the appearance of many new granules. This was first clearly stated by DARWIN in his studies of *Drosera rotundifolia* published in 1875. After showing that ammonium carbonate is very effective in producing what he calls an "aggregation" of the protoplasm of the leaves, he goes on to show that in the cells of the roots, ammonium carbonate caused a cloudiness of the clear fluid which these cells contained, so that this fluid came to look "like a mezzo-tinto engraving". In later studies, DARWIN '82 showed that ammonium carbonate in solutions of 0.1 to 0.7 % caused the formation of many granules in the root cells of various plants, including for example, *Euphorbia peplus*. Even solutions of 0.01 % were effective. Apparently DARWIN saw the granules in the cell sap, rather than in the protoplasm. PFEFFER '86 extended DARWIN's experiments. He describes the formation of granules in the *cell sap* of the root cells of *Azolla*, *Euphorbia peplus*, *Ricinus* following exposure to solutions of ammonium carbonate, sodium carbonate, or potassium carbonate. He also saw similar granules appear in the vacuoles of *Spirogyra* cells. BOKORNY '88 states that the granules appear not only in the vacuole, but in the protoplasm of *Spirogyra*, and he shows many figures of granules produced following treatment with ammonium carbonate. When 1 % ammonium carbonate was used, the granules formed very rapidly. They appeared more slowly when solutions as dilute as 0.005 % were used.

KLEMM '95 showed that alkalis caused the formation of numerous vacuoles within plant cells. There is also at least one older observation of the same sort (COHN '54), and there are probably others. DEGEN's studies of the phenomenon are especially

interesting (DEGEN '05). He found, for example, when the mycelium of *Aspergillus niger* was treated for 10 to 15 minutes with 0.1 % NaOH, that there was a production of so many vacuoles within the protoplasm that the whole mass of the protoplasm became foam-like in appearance. Similar results were obtained for the mycelia of *Mucor stolonifer*, *Dematium pullulans*, and *Saprolegnia*. For these forms, concentrations of 0.02—0.05 % NaOH were used. DEGEN also describes the formation of vacuoles following treatment with alkalis in the root tips of *Vicia faba*, in the pollen mother cells of *Lilium candidum*, in the root hairs of *Trianea* and *Helianthus*, in the gland hairs of *Syringa* and *Corylus* and in the hairs of *Urtica*. YAMAHA '27a also describes the appearance of vacuoles following treatment with alkali. He experimented on the root tips of *Vicia faba* and other plants, but he studied only fixed material.

ADDOMS '23 studied the action of solutions of varying pH on the root hairs of wheat seedlings. In her solutions, the acidity was due to phosphoric acid. The root hairs were examined in the darkfield with the aid of a cardioid condenser, and the time of exposure was a week. ADDOMS determined coagulation from the general appearance of the protoplasm in the darkfield. She states that there is no evidence of coagulation in solutions of pH 3.85 and 3.68, but at pH 3.60 and 3.47 she believes coagulation to occur. ADDOMS' results are consistent with the older observations KAHLBERG and TRUE and of HEALD (see above).

Attention should perhaps be called to the fact that there are a number of papers in the botanical literature which deal with the effects of acids and alkalies on growth. Thus LLOYD '18 showed that bean pollen grew faster when either acid or alkali was added to the culture medium. Other authors have shown that when the rate of growth of seedlings or roots is plotted against pH, a curve with two maxima is obtained (for literature consult ROBBINS '23). ROBBINS believes that the minimum between the two maxima represents the isoelectric point of the protoplasm. The growth of seedlings or roots is a complicated process and can hardly be expected to depend on any one physical property of the protoplasm. The entrance of water into the seed must depend to some extent on the colloidal properties of the seed coat. Doubtless the lifeless cell wall of the plant tissue plays a role, and there is also the plasma membrane to be consi-

dered. Moreover it is hard to see how one can exclude the chemical processes of metabolism from consideration. These are certain to be variously affected by differences in concentration of acid and alkali. In attempts to determine the isoelectric point of protoplasm, it should also be clearly understood that the pH of the interior of the cell is not the same as the pH of its environment.

In resumé, it may be stated that there is general agreement that acid in sufficient concentration causes coagulation of the protoplasm. Various observers note an increase in the granules of the cell as the hydrogen ion concentration is increased (KLEMM, LEWIS, PRÁT, STRUGGER). The coagulation produced by acid appears to be reversible, at least in some instances (LEWIS, VAN HERWERDEN). There is also some evidence that at hydrogen ion concentrations below those which cause coagulation there is a decrease in viscosity of the protoplasm as compared with neutrality. JACOBS found a decreased viscosity following treatment of protozoa with carbonic acid. PRÁT's observations indicate an increased fluidity of the protoplasm of onion cells following acid treatment. There is also a possibility that the increased rate of streaming in *Elodea* cells in acid solutions (ROBBINS) may be due to viscosity differences.

The liquefying action of acids, when it occurs, may be due to a fat solvent action of carbonic acid, either present originally or produced by a reaction of acid with carbonate, or it may possibly be due to a direct effect of the hydrogen ion in increasing the electric charge at the surface of the granule. It would then act like the calcium ion or the aluminum ion (see chapter 9). Or, if the protoplasmic granules are protein, and are not surrounded by a lipid film, the addition of hydrogen ion would act on the hydrogen ion dissociation of the amphoteric protein molecule, and this would result in a change in the charge on the granules. Unfortunately there are too many possibilities.

Regarding the action of alkalies, there is a difference of opinion, due possibly to a difference of material. LEWIS believes that in tissue culture cells, alkalies produce liquefaction. It must be confessed however that her evidence is not very good. PRÁT's observations indicate an increase in the viscosity of the protoplasm of onion cells following treatment with dilute alkali. The older observations of DARWIN and of BOKORNY indicate congu-



lation of protoplasm following exposure to dilute solutions of alkalies.

It is obvious that our knowledge regarding the action of acid and alkalies on the colloidal properties of protoplasm is far from satisfactory. There is a real need for careful and comprehensive experiments on various sorts of material with different acids and alkalies.

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## CHAPTER XII

# THE ACTION OF FAT SOLVENTS

Protoplasm is extremely sensitive to fat solvents. Very dilute solutions of ether and chloroform have a profound effect on the life of the cell. All fat solvents are anesthetics; they tend to repress or inhibit the activities of the living substance. The theory of anesthesia will be considered in a later chapter. For the present we are concerned with the physical effect of fat solvents on protoplasm, quite apart from any relation this might have to the life of the cell.

Beyond any question the action of fat solvents is many-sided. In the first place, all fat solvents are liquids of low surface tension. Even in great dilution, their aqueous solutions have a surface tension decidedly lower than that of water. When living cells are placed in such solutions, the boundary surface between the cells and the surrounding fluid has its surface tension lowered. If the cell is surrounded by a relatively thick membrane, there is also a lowering of surface tension at the boundary between this membrane and the inner protoplasm. The result of these surface tension lowerings is that the cell is no longer subjected to so great a pressure from without, the equilibrium between the various osmotic, elastic and surface forces no longer holds, and the cell tends to expand. Obviously the effect is greater in those cells in which surface tension forces plays a larger rôle, that is to say in smaller cells\*. But even in cells as large as sea-urchin eggs, the diameter of the cell can be seen to increase very markedly when the eggs are placed in solutions of low surface tension. The same effect can also be noted in large protozoa, for example in paramecium.

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\* The internal pressure due to surface tension varies inversely with the square of the radius.

It seems logical to assume that the increase in volume of cells which occurs in solutions of fat solvents is actually a direct result of the surface tension lowering. One fact speaks against such an interpretation. As the concentration of the fat solvent is gradually raised, there is a more or less definite point at which the increase in volume suddenly occurs. Before this concentration is reached, there is often little or no effect. Thus *Arbacia* eggs expand scarcely at all in 2% ether, whereas in 3% ether they very quickly undergo a large increase in volume. The surface tension of a 3% ether solution (against air) is not very different from that of a 2% solution. One would expect therefore, that as the concentration of the ether is raised, that there would be a marked increase in volume at first and then a more gradual increase. Perhaps the reason why this does not occur is as follows. The *Arbacia* egg is surrounded by a rigid or semi-rigid membrane. The rigidity of this membrane offers resistance to an increase in volume of the cell. As the surface tension is lowered, there is first not enough of an effect to overcome the rigidity of the membrane. When higher concentrations of fat solvent are used, there is suddenly a point at which the rigidity of the membrane is no longer sufficient to keep the volume small, and the cell increases in size quite suddenly\*.

This explanation is not entirely convincing, but whether or not it is correct, it is at least certain that in higher concentrations of fat solvent, cells very often undergo a marked increase in volume. This is a factor that sometimes becomes of importance in the interpretation of the behavior of protoplasm toward fat solvents.

Many authors have studied the action of fat solvents on gelatin and other proteins, and their results have often been taken as valid for protoplasm. With the development of the first methods for the examination of the colloidal behavior of protoplasm, it soon became apparent that protoplasm in no case acted like any simple protein, and that particularly in its relation to fat solvents was the living substance unique.

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\* There is also another possible explanation of the increase in volume of cells exposed to fat solvents. Perhaps the fat solvent releases bound electrolytes, (compare ROAF and ALDERSON '07), and in this way increases the internal osmotic pressure. Against this explanation is the fact that substances like toluol which apparently do not enter the cells, also cause increase in volume.

In recent years, determinations of protoplasmic viscosity in various types of animal and plant cells have shown conclusively that dilute solutions of fat solvents cause a decrease in viscosity, whereas somewhat more concentrated solutions produce coagulation. These facts were already suggested in the older literature of protoplasmic streaming. Thus KLEMM '95 states that when alcohol is gradually added to a slide preparation of the leaf of an aquatic plant, there is a marked acceleration in the rate of protoplasmic flow. Only when a relatively high concentration of alcohol is reached, does the flow become slower, until it gradually ceases. Following KLEMM, various other observers showed that dilute concentrations of fat solvents had an accelerating effect on protoplasmic streaming, whereas higher concentrations had an opposite effect. Thus EWART '03 states that when alcohol is gradually added (so as to avoid shock) to *Chara*, *Nitella*, *Elodea*, *Vallisneria*, or *Trianea* protoplasm, there is an increase in rate of streaming in concentrations of 1 to 2 percent. At stronger concentrations the rate of flow is retarded or stopped. Similarly, according to EWART, dilute solutions of ether and chloroform accelerate streaming, whereas stronger concentrations retard or stop it. JOSING '01 also reported observations of this sort. VORCK '10 studied the rate of streaming in myxomycete protoplasm. He too found that dilute ether solutions caused an increase in rate of streaming and that more concentrated solutions slowed or stopped it. Botanists have usually thought that the acceleration of protoplasmic streaming by dilute solutions of ether is the result of a stimulation, such as is sometimes thought to occur in animal tissues exposed to dilute solutions of anesthetics. It has therefore been more or less generally supposed that the effect of the fat solvents was on the motive force of the streaming. However, in view of the results that we shall consider below, it is much more likely that the increased speed of protoplasmic flow following treatment with dilute solutions of fat solvents is due more to a decrease in viscosity than to any stimulation of the driving force of protoplasmic streaming.

The first definite viscosity measurements of protoplasm treated with fat solvents were made by HEILBRONN '14. Using the gravity method, he found that in the starch sheath cells of bean plants, dilute solutions of ether produce a decrease in viscosity, more concentrated solutions an increase in viscosity. It

is hard to tell exactly which concentrations of ether HEILBRUNN used, for he gives his figures in percents of a concentrated aqueous solution rather than in actual percents of ether. In HEILBRUNN's experiments, both the state of decreased viscosity and the state of increased viscosity were reversible.

WEBER '22b repeated the earlier work of HEILBRUNN on bean cells, paying particular attention to the higher concentrations of ether. He studied the starch sheath cells of *Phaseolus vulgaris*, and made viscosity determinations with the centrifuge rather than the gravity method. His results show that  $2\frac{1}{2}$  and 5% ether (by volume) cause a marked increase in viscosity when the cells are exposed for an hour. The viscosity increase is at least twelve-fold, as shown by the relative times necessary to cause movement of starch grains in etherized and control cells. The viscosity increase produced by  $2\frac{1}{2}$ —5% ether is reversible. Exposure to 10% ether produced a more pronounced increase in viscosity which was irreversible.

HEILBRUNN '17, 20a, 20b, studied the effect of ether and other fat solvents on the protoplasm of sea-urchin eggs. Using the centrifuge method, he was able to show that various fat solvents caused a decrease in viscosity when present in certain dilute concentrations. In somewhat higher concentrations, they produced a coagulation with a great increase in viscosity. The decrease in viscosity produced by dilute solutions of fat solvents was always reversible. On the other hand the coagulation caused by the more concentrated solutions was always irreversible and involved the death of the cell. In a later chapter it will be shown that the decrease in viscosity produced by the fat solvents is related to the anesthetic action these reagents have in preventing cell division in the sea-urchin egg. For the present we are concerned only with the physical action of the fat solvents, and not with any physiological correlations.

HEILBRUNN '20b published a list of fat solvents with the percents of solution which caused decrease in viscosity and coagulation in sea-urchin eggs. In this list no statement was made with regard to the length of exposure. This is really an important factor. Sometimes a solution which causes first a decrease in viscosity, on longer exposure causes coagulation. The list published in 1920 has therefore been amended in the following table, so as to include data on this point. In this table, the second column

shows the concentration of reagent found to cause a decrease in protoplasmic viscosity. The length of exposure to this concentration of reagent is given in the third column. In the fourth and fifth columns are given the concentrations found to produce coagulation with the length of exposure actually employed. All the data refer to the fertilized eggs of *Arbacia*. The percents given are volume percents.

Table 1

Reagent	Concentration of solution found to decrease viscosity %	Length of exposure in minutes	Concentration of solution found to cause coagulation %	Length of exposure in minutes
Ether . . . . .	2.5	9	3.5	40½
Chloroform . . .	0.13	6½	1 (emulsion)	8½
Chloral hydrate .	0.08	13	1	35
" " .	0.25	28	—	—
Nitromethane . .	2	24½	3	16
Paraldehyde . .	4	7, 12½	8	6½
Acetone . . . .	5	29	10	9
Ethyl nitrate . .	0.3	6½	—	—
" " .	0.5	29	—	—
Ethyl acetate . .	3	41½	5	19½
" " .	4	6½	—	—
Ethyl butyrate .	0.25	33½	0.5	5
" " .	0.33	18	—	—
Acetonitrile . .	4	6½	5	13½
Propyl alcohol(n)	1	10	—	—
" " " .	1.33	25	—	—
Amyl alcohol . .	0.66	28½	1	40
Phenyl urethane	⅔ sat.	6	saturated (= < 0.5%)	15
Ethyl urethane .	1.5	8	3	29½
" " .	2.5	18	—	—

The above table is little more than a list of scattered experiments, and it is presented here only because more accurate data are lacking. Such data are easy to obtain. It would be a simple matter to investigate much more thoroughly the effect of various fat solvents on the physical state of the protoplasm. Careful

study in this direction might yield many results of considerable value.

In a short statement published in 1924, CHAMBERS states that 2% ether does not produce a decrease in viscosity but rather an increase in the protoplasmic viscosity of sea-urchin eggs. He bases this opinion on observations of Brownian movement in etherized eggs, as well as on microdissection tests. However, as pointed out by HEILBRUNN '25, these observations of CHAMBERS are unreliable. In estimating the rapidity of Brownian movement, CHAMBERS employed an arc lamp for darkfield illumination and took no precaution to protect the eggs from heat. *Arbacia* eggs die at only a few degrees above room temperature, and as shown previously (see p. 120), their heat death is hastened by ether. Doubtless in his estimates of viscosity with the microdissection needle, CHAMBERS was influenced by his studies of Brownian movement in the heat-coagulated eggs, and was thus led to conclude a higher viscosity when there was actually a lower viscosity.

Because of the above-mentioned statement of CHAMBERS, HEILBRUNN '25 reexamined the question of the effect of ether solutions on the protoplasmic viscosity of sea-urchin eggs. Both the unfertilized and the fertilized eggs of *Arbacia* were studied. The following table shows the action of 2½% and 3% ether solutions (in sea-water) on the protoplasmic viscosity of unfertilized eggs. The time of exposure, as given in the second column, is important, especially for the experiments with 3% ether. When eggs are exposed to this concentration of reagent, the viscosity of the protoplasm decreases gradually, passes through a minimum, and then after about half an hour, the stage of low viscosity is followed by an irreversible coagulation.

The variation of the viscosity of the control eggs in Table II. is due to the fact that there was some difference in the criteria employed in determining viscosity in different experiments. When the values in the table are averaged, it is found that 2½% ether reduces the viscosity of unfertilized *Arbacia* eggs to 53% of its original value, whereas 3% ether reduces the viscosity to 48% of what it is in the controls. No great claim for accuracy is made for these figures. It is nevertheless certain that dilute ether solutions do cause a very pronounced decrease in viscosity.

Table II

Concentration of ether solution %	Exposure in minutes	Relative viscosity of etherized eggs	Relative viscosity of control eggs	Temperature ° C.
2.5	11	10	25	23
2.5	4	15	25	—
2.5	8	15	25	22
2.5	10	15	28	24
3	15	15	35	22
3	8½	20	40	23
3	3	15	30	25.3

The effect is even greater when the experiments are performed on fertilized eggs. Following fertilization in the sea-urchin egg, there is a sharp increase in viscosity (see chap. 15). When fertilized eggs of *Arbacia* are placed in 2 ½ % ether at a time when the viscosity of the protoplasm is at its height, the viscosity soon becomes lowered to one-sixth or even one-eighth of its original value.

Both in the starch sheath cells of the bean plant and in the eggs of the sea-urchin, dilute concentrations of fat solvent produce a decrease in protoplasmic viscosity, whereas somewhat greater concentrations cause viscosity increase or coagulation. These results are not peculiar to the two types of cells mentioned. Various authors have obtained similar findings for other sorts of protoplasm.

HEILBRONN '22 performed some experiments on the effect of ether on plasmodia of the slime mold *Reticularia*. As a result of these experiments, he concludes that "schwache Narkose erhöht, wie früher für *Vicia faba* festgestellt, auch bei *Reticularia* die Liquidität, starke die Zähigkeit". In other words low concentrations of ether decrease the viscosity, higher concentrations increase it. Because of the fact that direct contact with ether solutions apparently injured the slime mold, HEILBRONN exposed the plasmodia on a moist glass slide to ether vapor. With such a procedure it is hard to regulate the exact amount of ether to which the protoplasm is exposed. Because of the uncertainty of the conditions of the experiment, we shall not cite HEILBRONN's



observations in detail. His general conclusion, as given above, is surely justified.

In 1921, WEBER ('21c) published a long series of experiments on the effect of ether solutions on the cells of the alga *Spirogyra*. The viscosity was tested by the centrifuge method. WEBER's results are very clear and convincing. With weak concentrations of ether, that is to say concentrations of 1—2½ %, there is a diminution of viscosity when the filaments are exposed 1—2 hours; with higher concentration (about 3 % or over), there is an increase in viscosity. The percents are volume percents. At the "critical" concentration of 2½—3 %, the effect varies with the time of exposure. A one hour exposure causes a decrease in viscosity, whereas an exposure of 3 or more hours has the opposite effect.

These results of WEBER show that *Spirogyra* cells behave toward ether concentrations of one to three percent in almost the identical fashion that sea-urchin eggs behave. The similarity of concentrations and times of exposure required to produce decrease and increase of viscosity in the two widely different types of protoplasm is truly remarkable. There is, however, one difference that the plant cells show. In sea-urchin eggs only the decrease in viscosity is reversible, the increase in viscosity produced by higher concentrations of the fat solvent is associated with death. On the other hand, the *Spirogyra* cells can also undergo a reversible increase in viscosity, although with concentrations of ether higher than 3 %, the viscosity increase is just as irreversible as it is in sea-urchin eggs. The difference between the plant and animal cells may in part at least be occasioned by the presence of the cell wall around the plant cells. When a sea-urchin egg is exposed to a relatively concentrated solution of a fat solvent, it undergoes a change sometimes called cytolysis, and this cytolysis is often associated with a marked increase in the volume of the cell. Such a volume increase would be prevented by the cell wall of plant cells.

In a later paper, WEBER '24d showed both by the plasmolysis-form method and by centrifuge tests, that a half hour treatment with 10 % alcohol has a pronounced liquefying action on the protoplasm of *Elodea* cells. Using the plasmolysis-form method again, he was able to demonstrate a decrease in the protoplasmic viscosity of the stalk cells of *Callisia repens*

(one of the *Commelinaceae*), following an hour's exposure to 2 % ether.

Finally BĚLEHRÁDEK '24-'25 has described a viscosity decrease in the protoplasm of *Elodea* cells, following treatment with chloral hydrate. However the method used by BĚLEHRÁDEK is open to serious question (see p. 51). BRINLEY '28 b reports an increase in Brownian movement when ameba is exposed to ether, chlorolorm, or alcohol.

The action of fat solvents has been carefully studied in the starch sheath cells of bean plants, in sea-urchin eggs, in slime mold plasmodia, in the leaf cells of *Elodea*, and in the stalk cells of *Callisia repens*. The results on all these different types of protoplasm are fully concordant, and it can now be regarded as scientific fact that, in general, dilute solutions of fat solvents cause a decrease in protoplasmic viscosity, whereas more concentrated solutions cause increase in viscosity or coagulation.

It should perhaps be stated that ETTISCH and JOCHIMS '27 have examined under darkfield illumination isolated frog nerve fibers treated with various alcohols. They conclude from their studies that ethyl, methyl, and propyl alcohol in dilute concentration cause a coagulation of the interior protoplasm of the nerve fiber. It has already been pointed out that the darkfield method is not applicable to the study of the interior of the nerve fiber (see p. 159). It might also be mentioned that ETTISCH and JOCHIM's results with ethyl alcohol do not accord with well known physiological facts. ETTISCH and JOCHIMS report a coagulating action with ethyl alcohol solutions of 0.3 to 0.9 % by volume. But many observations in the literature show that much higher concentrations of alcohol have no deleterious effect on intact nerves. Thus, to quote only one or two instances, LEE and SALANT '02 found that when they injected alcohol up to 1.58 % of the weight of a frog, i. e., about 2 % by volume, there was no evidence of injury either in nerve or muscle. In these experiments, the nerves of the frog were almost certainly bathed in a solution of alcohol of about 2 %. FOLGER '27 raised tadpoles in 1 % ethyl alcohol and kept them in this solution indefinitely. Many other observations of the same sort could be cited. It seems certain that ETTISCH and JOCHIM's results are due either to an error in method or to the fact that the fibers they studied were in a dying condition.

Before leaving the subject of the action of fat solvents, and before considering the possible explanation of the peculiar effects produced by these reagents, we shall make brief mention of the effect of ordinary distilled water on protoplasmic viscosity. This discussion is not out of place at this point, for it seems probable that water alone does actually exert a solvent action on the fats of living cells. Thus HANSTEEN CRANNER '22 describes a solution of the fats or lipoids of the outer protoplasmic layers of plant cells when these are treated with distilled water. Moreover when sea-urchin eggs are exposed to solutions containing a high percentage of distilled water, there is apparently a disappearance of the fat particles which ordinarily show so plainly when the egg is centrifuged.

When *Arbacia* eggs are treated with diluted sea-water, the protoplasmic viscosity is decreased, but when the dilution with distilled water becomes too great, there is a coagulation. This is clearly shown in the record of the following experiment, which is one of a series of similar experiments performed during the summer of 1925. Eggs were placed in mixtures of sea-water and distilled water, containing respectively 25 %, 40 %, 50 %, 60 %, and 75 % distilled water. Centrifuge tests were begun 10 minutes after the exposure to the hypotonic solution started, and an effort was made to complete each series of tests within 5 minutes. This was not always possible, but the viscosity values obtained from these tests can be regarded as valid for a 15 minute exposure to the hypotonic solution. In making the tests, the number of seconds required for the first appearance of a hyaline zone free from granules was taken as a measure of the viscosity. The centrifugal force used was 4968 times gravity. In the more dilute solutions, the eggs tended to break up when centrifuged. In some of the tests of eggs exposed to these more dilute solutions, the centrifuge was turned at lower speed, and the viscosity values were then recalculated on the basis of the higher speed. Because of the rapidity with which the tests were made, they are not especially accurate. The viscosity values are given in the following table. The temperature of the experiment was 23.5° C.

Table III shows the relative viscosity after an exposure of approximately 15 minutes. If the exposure to the hypotonic solution is 30 minutes instead of 15 minutes, then coagulation occurs in 60 % distilled water.

Table III

Percent distilled water	Viscosity
0	15
25	7
40	5
50	5
60	5
75	more than 60, probably infinity

But in these determinations of the viscosity of the protoplasm of cells in hypotonic solutions, there is a large source of error which must now be considered. In the hypotonic solution the cell increases in volume, and the distance between the adjacent granules accordingly becomes greater. In a previous discussion, (see p. 58), it has been shown that the ease with which the granules move through a cell depends in part on the distance which separates them from one another. CUNNINGHAM's factor, which we have called  $q$ , is less as the granules are farther apart. In order to determine the effect of this change in CUNNINGHAM's factor,  $q$  was calculated for the case in which the eggs are in 25 % distilled water, i. e. 75 % sea-water solution. For method of calculating  $q$ , see Chap. 5. As a result of the calculation,  $q$  was found to be approximately 8.1, that is to say 77 % of what it is when the eggs are of the normal size. Correction for the viscosity value obtained for 25 % distilled water shows that the proper value should be 9 instead of 7. The viscosity of the protoplasm of the *Arbacia* egg in 25 % distilled water is thus about 60 % of what it is in sea-water.

An interesting check of this method of calculation may be obtained by studying the effect of hypotonic solutions in the cold. At a temperature of about 4° C., the fat solvent action of the distilled water is much lessened, so that the main effect on the speed of granular movement under the influence of centrifugal force is apparently the change in  $q$ . In one experiment in which *Arbacia* eggs were exposed to 25 % distilled water for half an hour at 4°, the speed of granular movement at the end of this period was 71 % of what it was in the control eggs in sea-water.

From this experiment, it is certain that when eggs are placed in 25 % sea-water,  $q$  can not be less than 71 % of its original value\*.

It is certain, therefore, that when the protoplasm of the sea-urchin egg becomes diluted with distilled water, there is actually a pronounced decrease in viscosity. This is quite comparable to the effect of ether. In 25 % distilled water, the viscosity of the *Arbacia* protoplasm is about 60 % of its normal value. In  $2\frac{1}{2}$  % ether, it will be remembered, the viscosity drops to 53 % of what it is in the controls. Moreover, just as in higher concentrations of ether coagulation occurs, so too as the concentration of the distilled water increases beyond a certain limit, there is a sudden coagulation of the protoplasm.

It has repeatedly been shown that fat solvents cause a decrease in the viscosity of the protoplasm when they are present in relatively low concentration, whereas in higher concentration they cause coagulation. Obviously a most important question is the reason for this peculiar behavior of the protoplasmic colloid. This is a question which for the present we must leave unanswered. That dilute solutions of ether and other fat solvents should have such a pronounced effect on the protoplasmic viscosity certainly indicates that lipoids are a very essential part of the living substance. It seems not at all unlikely that the micellae of the protoplasmic colloid are surrounded by a lipoid film. Some evidence in support of this view has already been presented (see p. 30). But exactly how a dilute solution of a fat solvent would affect such micellae so as to decrease the viscosity of the whole colloid is by no means clear.

Concerning the coagulative action of more concentrated solutions of fat solvents, we now have some real information. This coagulative action can be seen morphologically to be accompanied by certain distinctive visible changes. In *Arbacia* eggs, for example, there is a very noticeable loss of pigment from the pigment granules of the cell, and this is soon followed by the appearance of numerous vacuoles throughout the protoplasm.

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\* It may be somewhat more, for in the cold the viscosity of the protoplasm tends to be lower than at room temperature, and this would make the relative value of  $q$  too low. But the effect of cold is more or less neutralized by fat solvents (HEILBRUNN '20a), and hence probably also by distilled water.

All these changes are associated with a very definite and characteristic series of reactions which are found not only in sea-urchin eggs, but in other cells as well. The fat solvent, when present in sufficient concentration, can initiate this series of reactions, and the ultimate result is a coagulation of the protoplasm. What is known concerning the nature of these reactions and how their initiation depends on the presence of fat solvent, will be discussed in a later chapter, (see Chap. 14).

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## CHAPTER XIII

# THE SURFACE PRECIPITATION REACTION

When a living cell is cut or torn, so that its interior flows out, a film is immediately formed at the surface of the emerging protoplasm. At any rate some cells behave in this fashion. The phenomenon is well known through the oft-quoted observations of NÄGELI '55 and PFEFFER '77. Many books refer to these observations as though they were practically the only ones on the subject. As a matter of fact, there are numerous similar observations scattered through the literature of the last hundred and fifty years. Merely from the standpoint of historical interest, it may be worth while to mention some of this neglected literature.

The early students of protozoa knew that when a protozoan was injured or crushed, the protoplasm might disperse through the surrounding medium. For the French workers, this was "décomposition par diffuence", the Germans refer to it as a "Zerfließen". But not always did a protozoan show diffuence. Sometimes, as the protoplasm began to emerge from the bounding membrane of the cell, it became coated with a film, so that a droplet or droplets were formed which were sharply delimited from the surrounding medium. These droplets were called by DUJARDIN "globules de sarcode". Following DUJARDIN, the Germans called them "Sarcodetropfen". They were seen by observers long before DUJARDIN. MÜLLER 1786 describes "sarcode droplets" in a species of *Colpoda*, and in an injured specimen of *Kerona*, he calls them "moleculae mucidae". The early observers did not know how to interpret the globular extrusions of protoplasm, and they usually thought of them as representing organs of one sort or another. EHRENBERG believed them to be stomachs. DUJARDIN gave the first sensible interpretation, (see for example DUJARDIN 1838). There can be no question but that the sarcode droplets of DUJARDIN and the similar appearances described by authors before and after him really represent the form of reaction

that we shall consider in the present chapter. One of DUJARDIN's figures is shown in Fig. 9. It is taken from his monograph published in 1841, and shows film formation around droplets of protoplasm emerging from *Leucophrys striata*, a protozoan parasite of the earth-worm. Following DUJARDIN, sarcode droplets were described and figured by various subsequent observers. These include FABRE-DOMERGUE '88, KÖLSCH '02.

Many students of protozoa, interested either in the function of the nucleus or in the capacity of parts of the cell to regenerate, have performed cutting experiments on many different types of single-celled organisms. In the course of these experiments, it has often been incidentally noted that the naked protoplasm

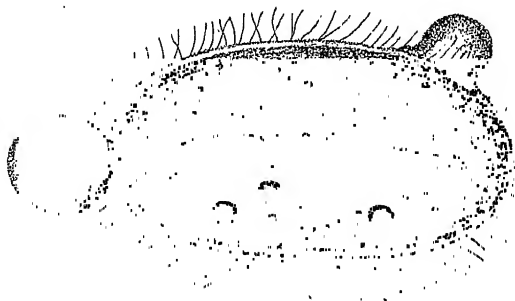


Fig. 9. The emergence of protoplasm from the cell of the protozoan *Leucophrys striata*, after DUJARDIN '41.

exposed by the cut was capable of covering itself with a surface film. Indeed it is probable that only in those cases in which such a film forms rapidly are cutting experiments possible. Modern books on protozoa contain many references to experiments in which different types of protozoa have been cut.\* Some of the early work is referred to by GRUBER '86, '93. From the first, ameba and *Stentor* were favorite forms for experimentation, and it is obvious that in these cells the naked protoplasm must quickly form a membrane or film.

Cutting experiments have not been confined to protozoa. Students of experimental embryology have frequently cut or

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\* See CALKINS '26, DÖRFLER and REICHENOW '27 (p. 329).



broken up the egg cells of marine invertebrates. Most of these experiments were performed on sea-urchin eggs. In the embryological literature, one finds very little said about the formation of a membrane or film at the surface of naked protoplasm. This is in part due to the fact that for a long time there was a difference in opinion as to the true structure of the sea-urchin egg. The outer membrane of the unfertilized egg has a refractive index close to that of sea-water and it is not very clearly visible. In the early part of the twentieth century it was generally believed that the sea-urchin egg was naked, in spite of the fact that previous workers had clearly described the egg membrane or vitelline membrane. Concerning the *Arbacia* egg, HEILBRUNN '15a states: "If the egg contents be made to flow out from the membrane or if the egg be cut or shaken into fragments, a new membrane immediately forms about the momentarily naked protoplasm. Such a membrane has the same chemical properties as the vitelline membrane."

It is much easier to break a large cell than a small one. This explains why practically all the observations on cut or torn cells have been made on protozoan or egg cells. There is, however, one old observation on small cells which is very interesting. HENSEN '61 crushed blood cells of the frog, and in one of his figures he shows the formation of a membrane or film about the droplet of exuded protoplasm. In view of our later discussion, it is worth mentioning that the emerging protoplasm from the blood cell loses its pigment. This is perhaps comparable to the loss of pigment from protoplasm pressed out of the *Arbacia* egg, (see below).

The literature on plant cells really begins with NÄGELI '55 although one finds occasional instances in which still earlier workers apparently noted the formation of a membrane or film about the surface of protoplasm squeezed out of cells, (see for example COHN '54). NÄGELI clearly describes the formation of a membrane about droplets of protoplasm which have emerged from *Chara* cells. He also studied the same phenomenon in the colored cells of grapes and other fruits.

Following NÄGELI, there is a long succession of workers who have described the formation of membranes about naked protoplasm. DE BARY '59 studied the behavior of naked slime mold protoplasm. HANSTEIN '72 (cited by STRASBURGER '76) described the formation of films about exuding droplets of *Vaucheria* protoplasm; see also HANSTEIN '80. KLEIN '72 cut the

mycelium of the fungus *Pilobolus* and noted the emergence of droplets. VAN TIEGHEM '75 notes film formation following wounding in the *Mucorineae*. STRASBURGER '76 describes the emergence of protoplasm from swarm spores of *Vaucheria sessilis*, and he says that a precipitation membrane is formed at the fresh surface. STRASBURGER also describes the formation of precipitation films on the freshly formed surfaces of plasmodia of *Aethalium septicum*. PFEFFER '77 crushed root hairs of *Hydrocharis* and saw films form at the surfaces of the emerging droplets of protoplasm. This work has been often quoted. SACHS '82 figures film formation around the naked protoplasm of a crushed filament of *Vaucheria* and he cites older observa-

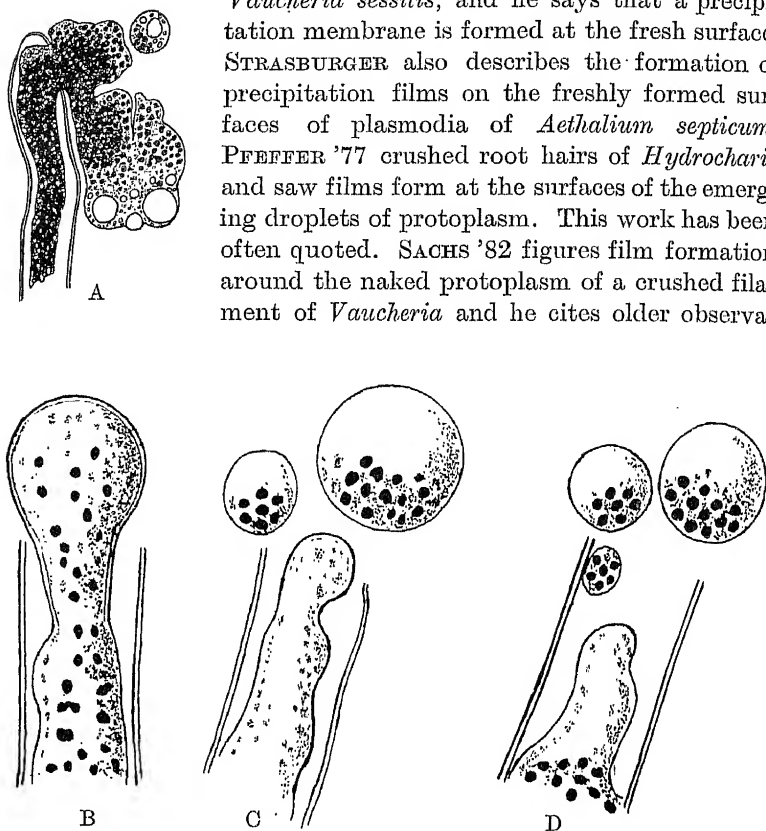


Fig. 10. The emergence of protoplasm from *Vaucheria* cells. A from SACHS '82. B, C, D, from HANSTEIN '80. B, C and D show successive stages

tions of SCHMITZ on *Valonia*. PFEFFER '90 made some observations on slime mold protoplasm. There have also been some newer studies; PROWAZEK '07 on *Vaucheria*, STÜBEL '08 on *Nitella*, SEIFRIZ '21 on *Rhizopus*, on eggs of *Fucus*, and on pollen tubes

of *Iris*, NICHOLS '22 on *Vaucheria*, *Cladophora*, *Chara* and *Nitella*, and LEFESCHKIN '26b on *Bryopsis*.

It is apparent that one of the favorite objects of study has been *Vaucheria*, and many figures have been published for this type of protoplasm. Fig. 10 is taken from SACHS '82 and from HANSTEIN '80. SACHS' drawing is especially interesting in that it shows the formation of vacuoles in the exuded protoplasm. We shall have frequent occasion later to refer to such vacuole formation. Fig. 11 is taken from PFEFFER '90 and shows the formation of a film or membrane at the cut surface of slime mold protoplasm.

In view of the large number of papers that have been written on the formation of membranes on the surface of protoplasm exposed in one way or another, it is rather surprising to find that some recent authors apparently regard the subject as new. Thus SEIFRIZ '21 and NICHOLS '22 write as if the first experiments were made with the microdissection needle.

In books on cytology and general physiology, it has often been assumed that the protoplasm of all cells forms surface films or membranes on exposure to the surrounding medium. Perhaps in a certain sense this may be true, but, as was known to MÜLLER and DUJARDIN many years ago, if one crushes various types of cells, one frequently finds that the protoplasm flows out through the surrounding medium without the appearance of any film or membrane. Thus, though very beautiful films may be obtained with *Stentor*, some races of paramecium show no film formation when the cells are pressed under a cover slip. In a few tests with *Colpidium*, it was found that usually when the cells were crushed under a cover slip, there was no appearance of a film about the emerging protoplasm. On the other hand, if the pressure to which the *Colpidia* were subjected was a very gentle one, films could sometimes be seen to form. As a matter of fact, even in those cells in which film formation appears most clearly, a very rapid extrusion of protoplasm may occur without any visible film being formed. It is possible that in

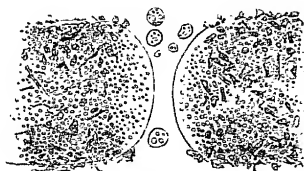


Fig. 11. Reaction at the cut surface of slime mold protoplasm, after PFEFFER '90

all cells films form if the protoplasm flows out at a slow enough rate, and that only in those cells in which the reaction is relatively rapid can it ordinarily be observed.

Many invertebrate eggs do not show film formation on being crushed. Thus tests made on the eggs of the molluscs *Cumingia* and *Ilyanassa*, and on the eggs of the annelids *Nereis*, *Chaetopterus*, and *Diopatra* failed to show film formation.\* However,

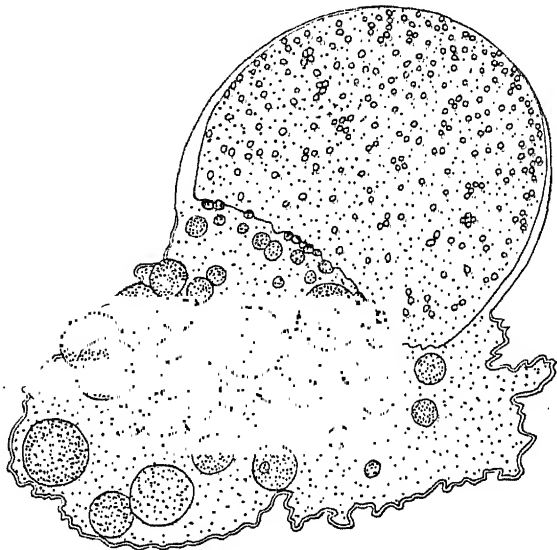


Fig. 12. The surface precipitation reaction in the egg of *Arbacia*, from HEILBRUNN '27 b.

BONFIG '25 describes film formation on extraovates from *Ascaris* eggs.

One of the prettiest objects for study is the egg of the sea-urchin *Arbacia*. When this egg is crushed under a cover slip so that the outer membrane is ruptured, the protoplasm which flows out soon becomes surrounded by a clearly visible film. In Fig. 12, this film or membrane can be seen surrounding the exuded protoplasm. Note also in this figure that in addition

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\* But more recent experiments show film formation in some of these eggs.

to the film at the outer surface, there are a number of internal films, which appear as vacuoles. These internal films or vacuoles vary greatly in number. When an egg is very gradually compressed, so that the protoplasm emerges slowly, there may be few or even no vacuoles. With greater speed of emergence, the number of vacuoles increases. Apparently their formation depends in part at least on whether or not the protoplasm in the interior of the emerging drop comes into contact with the surrounding medium.

What is the mechanism of the film formation on drops of naked protoplasm? For years the commonly accepted view has been that the film is the result of an accumulation of surface-active substances at the outer surface of the emerging protoplasm. It is certain that protoplasm contains substances which lower surface tension; in accordance with the GIBBS-THOMSON rule these would accumulate at a surface, just as peptone accumulates at a water-air surface and forms a solid film or membrane there. This explanation at first glance seems self-evident, and it has been accepted by practically every authority on cytology or cellular physiology. Until recently, it was never examined experimentally.

On the face of it, the comparison of protoplasmic films with the adsorption films which form at the surface of peptone solutions is not an accurate one. Peptone (and other surface active substances) become concentrated at the surface of water and air, or water and oil. But when protoplasm is cut, there is no surface tension surface between it and the surrounding watery medium. This follows from the fact that protoplasm itself has water as a dispersion medium. When an aqueous solution is brought into contact with pure water or with another aqueous solution, there is no surface film at the boundary. Hence if we are to assume that the film which forms around emerging droplets of protoplasm is an accumulation of the surface active constituents of the cell, then we must first imagine some sort of surface for these constituents to accumulate at.

If protoplasm were a gel, we might imagine a surface to exist between it and pure water. But surely it has been abundantly proven that the protoplasm of the sea-urchin egg is not a gel, (see chapters 5 and 6), and yet in this very instance film formation is especially easy to observe.

The idea that the films which form about naked protoplasm are adsorption films can also be shown by experiment to be

untenable. As is well known, adsorption occurs more readily in the cold. With a rise in temperature, surface tension decreases, and there is then less tendency for surface active materials to become concentrated at a surface. On the basis of the old theory, cold, since it favors adsorption, should favor the formation of protoplasmic films. Experiment shows it to have the opposite effect. When sea-urchin eggs are crushed at temperatures a degree or two above freezing, usually no film forms about the protoplasm as it emerges. Sometimes an incomplete film makes its appearance, if the pressure on the cover slip is very gentle. But it is readily apparent that low temperatures retard rather than favor the process.

The films which form about naked protoplasm are not adsorption films. As a droplet of fluid protoplasm streams out of a cell, some sort of a coagulation process must occur at the boundary of the droplet to prevent its diffusion throughout the surrounding medium. The entire process or processes which are involved in the formation of a film on the surface of exposed protoplasm have recently been called the "surface precipitation reaction", (HEILBRUNN '27b).

Experimental study of the surface precipitation reaction has shown that in various cells (*Stentor*, *Arbacia* eggs, *Echinarrachnius* eggs), the presence of calcium is a necessary prerequisite. If *Arbacia* eggs are washed in isotonic sodium chloride solution for a minute, and are then transferred to a second dish of isotonic sodium chloride, they no longer show any reaction when the cells are crushed. The protoplasm streams out through the sodium chloride solution without the formation of any film. A similar result can be obtained if the eggs are crushed in calcium-free sea-water. This experiment can be simply performed by adding a small drop of m/4 ammonium oxalate solution to a drop of sea-water containing eggs. After an interval of a minute or less, the eggs on being crushed show no surface precipitation reaction. Apparently a certain amount of calcium is necessary for the reaction to proceed. It should be noted that washing for a minute in isotonic sodium chloride solution, or treatment for a minute with oxalated sea-water can scarcely remove the calcium from the egg interior. Perhaps the calcium within the egg is not in an available form. This is a point which will be brought up again later.

It is probable that in ameba also the surface precipitation reaction depends on the presence of calcium. At any rate one may perhaps so interpret the experiments of CHAMBERS and REZNIKOFF '26. These workers tore amebae with microdissection needles. When the amebae were torn in dilute calcium chloride solutions, there was an immediate repair of the torn surface. Such a repair did not occur in dilute sodium chloride solutions. But CHAMBERS and REZNIKOFF found also that the repair that they describe took place in distilled water, supposedly in the absence of both sodium and calcium. From the effects of distilled water on sea-urchin eggs, (see next chapter), it seems possible that distilled water may free calcium from the interior of the ameba. In the normal movement of an ameba, the internal protoplasm may become exposed to the outer medium and form a new film about itself. Perhaps in this normal movement, the surface precipitation reaction plays a part. It is interesting to note that in the case of a marine ameba PANTIN '26 found no movement possible in the absence of calcium. See also POLLACK '28.

GRAFE '22 (see p. 47) states that the exuding protoplasm of *Nitella* rapidly forms a film about itself, but that this reaction does not occur when the protoplasm flows out into a dilute soap solution. Perhaps the effect of the soap is to remove calcium. Whatever our interpretation of these experiments of others, it is certain that in sea-urchin eggs and in *Stentor*, the surface precipitation reaction can not take place in the absence of free calcium.

The magnesium ion can not take the place of the calcium ion. Eggs washed with magnesium chloride solution do not give a surface precipitation reaction. On the other hand, the presence of small quantities of strontium permits the reaction to proceed. In other words, strontium can replace calcium.

Our experimental analysis of the surface precipitation reaction has established the fact that calcium plays an important rôle in at least two widely different types of protoplasm. Further study may show that the presence of calcium is an essential factor for the reaction in all types of living material in which it occurs.

In the sea-urchin egg, the pigment granules of the egg interior also play a part in the surface precipitation reaction. Fig. 12 shows that as the protoplasm of the egg emerges from its con-

taining membrane, the pigment granules disappear. Their color diffuses throughout the entire extraovate. When eggs are crushed in the absence of calcium, there is no surface reaction and no breakdown or disappearance of pigment granules. This observation led to the idea that perhaps the pigment granules were directly involved in the surface precipitation reaction, unlikely as such a participation might seem. The idea was soon tested. Eggs were centrifuged and were then crushed. In those eggs in which the cell membrane ruptured at the heavy end of the egg, where pigment granules were present, an excellent surface precipitation reaction occurred. When the rupture was at the opposite hemisphere, there was no reaction in the absence of pigment granules. But the failure of the reaction to take place might be due either to the lack of pigment granules or to the lack of the small colorless granules, for these are also absent from the light end of the centrifuged egg. Another test was then made in which eggs were first centrifuged and were then allowed to stand for 45 minutes before being crushed. Within 45 minutes, many of the colorless granules return to the light pole of the egg, but the pigment granules for the most part remain near the heavy end. Under these conditions, when the protoplasm was forced out of the eggs by pressure on the cover slip, no surface precipitation reaction occurred in the absence of pigment granules, even though colorless granules were present. Of the granular constituents of the egg, it is the pigment granules and these alone that take part in the reaction.

Our analysis so far has shown:

1. That the surface precipitation reaction is retarded or inhibited by low temperatures.
2. That calcium (or strontium) is essential.
3. That, in the sea-urchin egg, the pigment granules play a rôle.

In studying any colloidal reaction of protoplasm, it is always of interest to compare it with the reactions which may be observed in test tubes. Most colloids show no specific relation to the calcium ion. But both in the coagulation of blood and the clotting of milk, calcium plays an important part. The fact that blood and milk are biological colloids makes this resemblance more interesting. The surface precipitation reaction also resembles blood coagulation in another respect. Both are retarded or



prevented by cold. Perhaps, too, the breakdown of the pigment granules in the surface precipitation reaction is to be compared to the breakdown of the blood platelets in blood coagulation. In view of these similarities, it was thought worth while to study the surface precipitation reaction from the standpoint of blood coagulation.

Unfortunately the literature on blood coagulation is in a very confused state. There are dozens of theories, many with distinct terminologies for various known and assumed substances. But this much at least seems clear. There are two, and perhaps more stages in blood coagulation. The first stage involves calcium, and results in the formation of a substance called thrombin. In this stage, or previous to it, the blood platelets break down. In the second stage, the thrombin produces a clot, i. e. fibrin, by combining with or acting upon fibrinogen, a protein of the blood plasma. Calcium plays no part in the second stage of the blood coagulation process, and thrombin can act in its absence. Clotted blood contains thrombin, both in the clot and in the serum which emerges from the clot. A drop of serum will therefore clot a fresh sample of blood in the absence of calcium.

It has been shown that calcium and pigment granules are involved in the surface precipitation reaction in the *Arbacia* egg. Might it not be possible that there is a still closer correspondence to blood coagulation and that the need for coagulation is limited to a primary stage, following which calcium is no longer necessary? Just as the student of blood coagulation prepares thrombin from clotted blood, so it might be possible to prepare a comparable substance from the cells which have undergone surface precipitations.

This possibility was easily tested. A thick suspension of sea-urchin eggs was shaken up vigorously with splintered glass, so that the eggs were broken up into innumerable fragments. To the broken-up eggs oxalate was added to remove calcium. When a drop of this preparation was added to a drop containing eggs in calcium-free sea-water, and the eggs were then broken by pressure, the emerging protoplasm showed a surface precipitation reaction. Thus the preparation of broken-up eggs contains a substance which can produce a surface precipitation reaction in the absence of calcium, although when this substance is not present calcium is necessary for the reaction. The similarity to

blood coagulation is close. In cells as in blood, there are two stages to the coagulation reaction. In the *Arbacia* egg, the first stage involves an interaction of pigment granules and calcium, the second stage does not require calcium. Does it require pigment granules? Apparently not, for several tests in which centrifuged eggs were placed in oxalated sea-water plus extract of broken-up eggs showed a definite surface precipitation reaction when the protoplasm was made to emerge from the light pole. If these experiments are correct, in the presence of broken-up eggs, a surface precipitation reaction can occur in the absence of calcium and in the absence of pigment granules. Thus the first stage of the surface precipitation reaction seems to consist of a reaction between calcium and pigment granules. The result of this reaction is the formation of a substance which can produce a surface reaction in the absence of calcium and probably also in the absence of pigment granules.

It has just been shown that there is a substance in a preparation of broken-up eggs which can produce surface films in the absence of calcium. Because of the apparent similarity to the phenomena of blood coagulation, it was proposed to call this substance ovothrombin. The name ovothrombin should be regarded as provisional for two reasons. In the first place, similar substances may very possibly be found in cells other than eggs. If this proves to be the case, "cytothrombin" might be a better term to use. Secondly, when a true understanding of blood coagulation emerges from the welter of present-day theories, it may be found that the ovothrombin of the sea-urchin egg more truly resembles some one of the numerous other substances now suffering from a multiplicity of names, than it resembles thrombin itself. But for the present, the name ovothrombin will suffice as a label for the substance which can cause a surface precipitation reaction in the *Arbacia* egg in the absence of free calcium.

In the literature on blood coagulation, there has been much discussion as to whether or not thrombin is an enzyme. In two important respects ovothrombin does not resemble typical enzyme preparations. It is not destroyed by boiling. Preparations of ovothrombin can be boiled for ten or fifteen minutes without losing their efficiency. If anything, they seem to gain rather than to lose in potency as a result of boiling. Secondly, ovo-

thrombin diffuses readily through parchment membranes. In preparing relatively pure preparations of ovothrombin, it was found convenient to boil the broken-up eggs and then to place them in a parchment dialyzer. The ovothrombin then passed through the dialyzer.

Ovothrombin is surface active, that is to say, it lowers surface tension. This is shown by the fact that in solutions of ovothrombin the surface film of the solutions shows greater evidence of activity, that is to say is more efficient in causing a precipitation reaction, than is the fluid in the interior.

Some preparations of ovothrombin were found to deteriorate after two or three weeks. It is destroyed by weak solutions of potassium permanganate. Moreover, in the only experiment that was tried, the addition of 0.2 cc. of  $n/100$  HCl to 1 cc. of an ovothrombin preparation caused a loss of activity of the latter after an hour and a half.

Although ovothrombin can cause a surface precipitation reaction in the absence of pigment granules, sometimes the pigment granules do break down when eggs are crushed in oxalated sea-water in the presence of ovothrombin. Thus, although in some experiments one sees a perfect surface precipitation reaction without loss of pigment on the part of the pigment granules, in other cases the initiation of a reaction by ovothrombin is accompanied by a fading of the pigment granules or even by their complete disappearance. Apparently the greater the concentration of the ovothrombin, the greater the tendency for the pigment granules to break down, but no accurate experiments were made to settle this point definitely. It seems certain that when ovothrombin initiates the surface precipitation reaction, the breakdown of the pigment granules is not a necessary preliminary, but is more in the nature of an after effect. Not infrequently the surface precipitation reaction occurs first, and the pigment granules then slowly break down or lose their pigment later. This suggests an interesting possibility. It may be that in the second phase of the surface precipitation reaction, calcium is again set free, and that it is this calcium which causes the breakdown of the pigment granule. If this were true, we might have the following scheme for the stages of the surface precipitation reaction in the *Arbacia* egg:

1.  $\text{Ca} + \text{pigment granule} = \text{ovothrombin}$

2. Ovoidthrombin + a protein = film or membrane + Ca.

If this scheme represents the true state of affairs, it is obvious that a small amount of ovoidthrombin can have a great effect. The reaction is self-propagating.

The fact that a living cell contains a substance which resembles thrombin need not be at all surprising. Many authors have held that extracts of various types of cells can have an accelerating effect on the coagulation of mammalian blood. Consult, for example, SCHMIDT '92. Not only cells of vertebrates, but even cells of plants have been found to yield extracts which hasten the coagulation of the blood of higher animals. It would be interesting to discover whether or not ovoidthrombin has any effect on the coagulation of the blood.

The fluid in the body cavity of a sea-urchin clots when it is poured out of the animal. It might be thought that this reaction would show similarities to the surface precipitation reaction of the egg cell, and that perhaps a thrombin could be isolated from sea-urchin blood which would have an effect on the surface reaction of the egg cell. But the clotting of sea-urchin blood is a very different phenomenon from the clotting of mammalian blood. It depends almost entirely on the clumping together of the blood cells. Apparently there is no thrombin produced, and the clotting occurs quite independently of the presence of calcium. One or two crude experiments were tried with human blood serum. It was not found to have any effect on the surface precipitation reaction in the *Arbacia* egg.

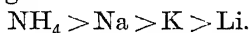
It has been shown that the surface precipitation reaction in the *Arbacia* egg can be separated into two stages, in the first of which calcium reacts in some fashion with pigment granules to form ovoidthrombin. This first stage of the surface precipitation reaction can be subjected to a closer analysis. We can seek to discover what sort of a process takes place when calcium and pigment granules are brought together. It is possible to remove the pigment granules from the cell interior and to study their behavior when they are in a known medium, rather than in the protoplasm. Ordinarily the pigment granules disappear when they are pressed out of a cell, but in the absence of calcium they remain intact. One has only to remove calcium from the surrounding medium in order to obtain a suspension of pigment granules. With such a suspension of pigment granules, the effect of various

reagents can be studied, and some observations have been made in this manner. However this method of study has its disadvantages. Pigment granules are only about a micron in diameter. They are of course easy to recognize either within the egg, or just after they have been pressed out of an egg. But in a suspension of broken-up eggs there are many small granules about a micron in diameter and not all of them are pigment granules. Nor is the color an absolute criterion that is easy to apply. Not only do the pigment granules fade somewhat, but also other granules apparently take on some of their pigment. It is much safer to identify the pigment granules just after they have emerged from the egg, when there can be no doubt as to their identification.

If eggs are placed in various solutions and are then crushed rapidly, one can compare the behavior of the pigment granules under various conditions. Many such experiments have been performed and some interesting results have been obtained. It is indeed possible to study the reaction between calcium and pigment granule outside of the cell. So far, although numerous observations have been recorded, the work is in a preliminary stage, and no satisfactory interpretation has been arrived at for some of the peculiar results. We shall, therefore, present only a brief sketch of this work, in order to indicate the type of results that can be obtained.

It has been stated that whenever a trace of calcium is present, the pigment granules break down. This is true under the usual conditions. But if eggs are placed in concentrated solutions of sodium chloride which also contain calcium chloride, then even in the presence of very considerable amounts of calcium, the pigment granules do not break down. With concentrations of sodium chloride up to one molar, very tiny amounts of calcium are sufficient to produce a disappearance of the pigment granules. But when the concentration of sodium chloride is raised to one and a half molar, much larger amounts of calcium can be added without any visible effect on the pigment granules. A curve may be plotted which will show the exact concentration of sodium necessary to prevent the action of calcium when it is present in varying amounts. Such curves are very interesting, especially when one notices the variations produced in them by the addition of reagents of one sort or another to the reacting system. Work of this sort is now in progress.

Sodium chloride is not the only salt that prevents the action of calcium on pigment granules. Ammonium chloride, lithium chloride, and potassium chloride all act in the same fashion. When one compares the action of these salts, it can be shown that the various cations are not equally effective. The following is the order of their efficiency in inhibiting the reaction between calcium and pigment granules:



This order is that of the lyotropic series. In studying the hydrolysis of an ester, ARRHENIUS\* found that ammonium salts exerted a greater inhibiting influence on the reaction than sodium or potassium ions. He also found that the more readily adsorbed anions had a greater inhibiting effect than the less readily adsorbed anions. So too in the *Arbacia* pigment granule reaction, the sulphocyanate ion tends to prevent breakdown of the granule more than does the chloride ion. There is thus a hint that the first stage of the surface precipitation reaction in the *Arbacia* egg may involve the hydrolysis of an ester. In view of the fact that cytoplasmic granules may very well be surrounded by a lipid film (see p. 30), the theoretical possibilities are interesting. Calcium by forming an insoluble soap and thus removing one of the products of the reaction, might favor the hydrolysis of a fatty substance. It is hardly worth while to push the theoretical discussion any further. We must wait for further experimentation. Fortunately the experiments are very simple to perform. We are really in a position to study a single reaction system of protoplasm outside of the living cell, but yet in definite relation to one of its morphological constituents. This is indeed a great opportunity.

It must not by any chance be thought that the reaction of pigment granule plus calcium is simple. In order to give an idea of the complexities, it may be stated that if eggs are crushed in concentrated calcium chloride solutions, there is no breakdown of pigment granules. This is indeed surprising. However, if the eggs are crushed immediately, within 5 seconds after entrance into the calcium solutions, there is a breakdown of pigment granules. Apparently in the concentrated calcium chloride solutions, or perhaps in any concentrated solutions, there is the formation

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\* ARRHENIUS 1887, Zeitschr. f. physik. Chem., vol. 1, p. 110.

within the egg of a substance which protects the pigment granules from breakdown. The fact that dilute calcium solutions favor the first stage of the surface precipitation reaction and concentrated solutions prevent it reminds one of the fact that dilute calcium solutions favor blood coagulation, but that more concentrated solutions retard it.

In the preceding pages we have emphasized the fact that in many respects the surface precipitation reaction of the sea-urchin egg resembles the blood coagulation of higher animals. But of course there must be differences. One such difference lies in the fact that whereas the coagulation of mammalian blood can only occur between fairly narrow limits of hydrogen ion concentration, the cell reaction is rather independent of the pH. KUGELMASS\* showed that blood coagulated only between pH 5 and pH 8. On the other hand, the surface precipitation reaction can occur at pH 3 and pH 10 and perhaps in even more acid and alkaline media. It should be remembered however that ovothrombin is destroyed by acid.

The student of blood physiology is faced with the problem of why the blood does not coagulate within the vessels, and he has proposed various types of explanation to account for the fact that the blood remains fluid as long as it stays inside the arteries. There is a similar problem involved in the surface precipitation reaction of living cells. Why does it not occur in the cell interior? Within the cell both calcium and pigment granules are present. We would expect that these would react, that the pigment granules would disappear and that films or membranes would be formed within the cell.

It seems probable that the surface precipitation reaction does not occur in the cell interior because of the absence of free calcium there. It has already been pointed out that when a cell is washed for a single minute in isotonic sodium chloride solution, that this treatment is sufficient to prevent a reaction when the cell is crushed. Treatment for a minute could hardly remove the calcium from the egg interior. We have reason to believe, therefore, that the calcium of the cell is not in a form available for the surface precipitation reaction. Perhaps it is adsorbed at the surfaces at some of the cell granules. If this were the case,

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\* KUGELMASS 1923, *Arch. Intern. de Physiol.*, vol. 21, p. 139.

then if we placed the eggs in an isotonic solution of calcium chloride, more and more calcium would enter the cell until at length there would be enough calcium to cause a breakdown of the pigment granules. This is actually what happens. When *Arbacia* egg cells are immersed in isotonic calcium chloride, at first there is a liquefaction of the cytoplasm, but then following an interval which varies greatly under different conditions, but which usually lasts several hours, there is a breakdown of pigment granules, and the egg interior becomes filled with numerous small vacuoles. Beyond much question these vacuoles are comparable with the vacuoles which can be seen when an egg is crushed under a cover slip and which are shown in Fig. 12. The wall of the vacuoles is doubtless the same sort of a film as the film which is produced in the surface precipitation reaction. By introducing additional calcium into the egg we have thus produced a surface precipitation reaction throughout the cell. Such a cell full of tiny vacuoles and with its pigment granules broken down presents a picture which since the studies of LOEB has usually been referred to as "pale cytolysis". If it be true that cytolysis in the sea-urchin egg is to be identified with a surface precipitation reaction throughout the cell, this is a very important conclusion, for it enables us to investigate the problem of such cytolysis from a new angle. This subject will be taken up in the next chapter.

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## CHAPTER XIV

# A SPECIFIC COLLOID CHEMICAL REACTION PECULIAR TO LIVING ORGANISMS

In this chapter an attempt will be made to bring together information concerning a reaction of the living protoplasm which has been studied by botanists, zoologists, physiologists, and pathologists. In its different forms it has been called a host of names at one time or another, and as a result workers in various fields have usually failed to recognize the extremely general nature of a phenomenon of which they have studied only a single aspect. If, in the pages that follow, it can be demonstrated that there is one essential reaction, which is fundamentally the same for many and perhaps all types of protoplasm, a great deal will have been accomplished. Often what is difficult or impossible to understand for a given type of cell is relatively simple for another. By bringing together all the information from various branches of the biological field, much can be gained.

The originator of the protoplasmic concept, DUJARDIN, was well aware of the tendency of protoplasm to undergo vacuolization, and indeed he regarded this as one of its essential, if not its most essential characteristic. In his first paper on protoplasm or sarcode, published in 1835, he says, "Mais la propriété la plus étrange du *Sarcode* c'est la production spontanée, dans sa masse, de vacuoles ou petites cavités sphériques". Later, in 1841, he writes that when he first defined the concept of sarcode, he felt that the peculiar property which living substance possessed of filling itself with watery vacuoles, was the very property which distinguished it most clearly from inanimate substances such as gelatin, mucus, or albumin.

Since DUJARDIN, no one has so strongly emphasized the vacuole-forming capacity of protoplasm. But there have been no lack of observations. Some of these we shall now proceed to consider.

If a living cell is placed in a hypotonic solution or in distilled water, it expands or at least tends to expand. This is common knowledge. But it does not seem to be so generally known that when a cell is placed in a sufficiently dilute solution or in water, vacuoles appear in the protoplasm. As a matter of fact, this phenomenon was known long before our present understanding of the osmotic behavior of a cell was arrived at. As it became increasingly evident that the laws of osmotic pressure constituted a satisfactory explanation of the expansion of a cell in water and hypotonic solutions, the older literature regarding vacuol $\acute{e}$  formation was forgotten. The botanists of the middle of the nineteenth century seem to have regarded the entrance of water into a cell as primarily due to the formation of watery vacuoles within the protoplasm. This view is hardly feasible now, but it is nevertheless of interest to know that there are numerous observations which show that water does cause vacuole formation in plant protoplasm. Who was the first to describe this phenomenon is not certain. In 1867, HOFMEISTER in his book on plant cytology, discusses the subject as though it were rather old and the facts well known. He says, "Die Entstehung und Ausbildung von Vacuolen ist direct zu beobachten an allen (von der gr $\ddot{o}$ sseren Dichtigkeit der peripherischen Schicht abgesehen) homogenen Protoplasamassen, welche in Wasser gelangen". Then he goes on to cite numerous examples, and he attempts a theoretical explanation of why entering water should cause vacuole formation in protoplasm.

WIESNER '69 in studying yeast cells noted that both when water entered the cell rapidly, and when it was made to leave rapidly by placing the cells in concentrated solutions, vacuoles appeared in the protoplasm. The fact that either a gain or a loss of water can cause vacuole formation is important. Compare also K $\ddot{U}$ STER '18.

Some years later, SCHWARZ '87 published some descriptions of the action of water on plant protoplasm. Figure 13 is taken from his monograph. It shows a young cell from the root tip of a pea seedling. Following treatment with water, the surface protoplasmic layer has become filled with small vacuoles. SCHWARZ also describes vacuole formation in the nuclei of some cells following exposure to distilled water, and there is moreover a vacuolization of chloroplasts.

Doubtless a thorough search of the botanical literature would bring to light many other descriptions of vacuole formation following treatment with distilled water. However, it is hardly necessary to multiply references. The fact is clear that distilled water does cause many vacuoles to form within plant protoplasm.

Many other agencies also cause vacuoles to appear in the protoplasm of plant cells. Unfortunately, no one seems to have been especially interested in the process of vacuole formation, so that in going through the literature one has to depend on sporadic observations made by various authors in the course of investigations on one subject or another.

Apparently fat solvents act like distilled water on plant protoplasm. NADSON and MEISL '26 describe the formation of vacuoles in onion epidermis cells following treatment with chloroform.

Both acids and alkalis cause a vacuolization of plant protoplasm. This was very carefully described by KLEMM '95, who studied a number of different types of material. See also DEGEN '05, and compare Chap. 11, p. 199.

When an electric current is sent through plant tissue, vacuoles often appear in the protoplasm of the cells. This phenomenon has been described by VELTEN '76b and by KLEMM '95. Radium radiations also cause a vacuolization of plant protoplasm (WILLIAMS '25, NADSON '25).

When plant cells are subjected to pressure or to mechanical injury, vacuoles appear in them. VELTEN '76b states, "Bei stärkerer Einwirkung des elektrischen Stromes, ebenso bei Druckwirkungen entstehen in den verschiedensten Zellen, wie dies bereits bekannt, Vacuolen". Unfortunately he gives no references to the older literature. NĚMEC '01b describes the formation of vacuoles in the cells of onion roots. When a root is cut or injured, vacuoles appear in the cells in the neighborhood of the cut, and

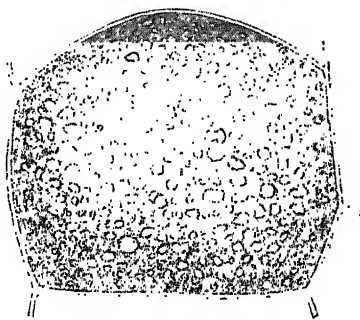


Fig. 13. Vacuoles in the protoplasm of a root cell which has been exposed to distilled water, after SCHWARZ '87.

there is a wave of vacuole formation which proceeds in all directions from the injured surface. HEILBRONN '21 describes vacuolization of slime mold protoplasm following mechanical injury. BUENNING '26b states that numerous vacuoles appear in onion epidermis cells as a result of mechanical coagulation.

Many authors have described vacuolization of plant protoplasm following exposure to high temperatures. These include GEORGEVITCH '10, HARTMANN '18, '19, WASSERMANN '21a, b; YAMAHARA '27b. Practically all of the work has been done on fixed material.

NABGELI '55 described the formation of vacuoles in degenerating plant protoplasm.

From our brief survey, it is apparent that distilled water, fat solvents (at any rate chloroform), acids and alkalis, mechanical injury, heat and the electric current all cause the appearance of vacuoles in various types of plant cells.

The literature on animal cells is more complicated, and we shall have to approach it from a number of different angles. In the first place, the observations on protozoan protoplasm are much like those which have been made for plant cells. SCHULTZE '63 describes the formation of vacuoles in the protoplasm of *Foraminifera* treated with distilled water. A similar observation has recently been made by LEPESCHKIN '25a. Passage of an electric current causes vacuoles to appear in amoeba (VERWORN '96b), and in paramecium (WALLENGREN '03, STATKEWITSCH '06). Radium treatment produces vacuolization in amoeba (ZUELZER and PHILIPP '25). Lack of food may induce vacuole formation in protozoa, WALLENGREN '02. As a matter of fact, vacuoles frequently appear in protozoan protoplasm under natural conditions. This is very apparent if one glances through BÜTSCHLI's monograph on the protozoa ('82—'89). Interesting figures of the extent to which this vacuolization may go are shown in a paper by SPEK '24a.

There have been occasional descriptions of vacuole formation in blood cells. FROMMANN '84 frequently refers to the appearance of vacuoles in the blood cells of crabs. BOETTCHER '66 describes the production of vacuoles in vertebrate blood cells treated with distilled water and with chloroform vapor. One or two other instances of vacuoles in blood cells will be mentioned later.

Vacuolization phenomena in the tissue cells of vertebrates have not infrequently been studied by pathologists. The literature

is not always easy to interpret. This is in part due to the fact that most of the work has been done on sectioned material, in part to the variety of terms used by different workers. VIRCHOW (see VIRCHOW '71) coined the expression cloudy swelling ("trübe Schwellung"), and since VIRCHOW's time this term has been a favorite one in the pathological literature. A cell which shows cloudy swelling is larger (and consequently paler), and it has in its protoplasm granules which have evidently been precipitated there. Apparently in animal cells as in plant cells (see KLEMM '95), there are two types of degenerative change, which may occur separately or together. One is the precipitation of new granules within the cytoplasm, the other is the formation of vacuoles there. Perhaps if the term cloudy swelling is to be specific, it should be restricted to those cases in which new granules appear in the cytoplasm. This appears to be in accordance with the original idea of VIRCHOW. Some recent authors, however, identify cloudy swelling with vacuole formation (see for example RUMJANTZEW '26a). The appearance of new granules in the protoplasm, in many cases at least, is due to the precipitation of protein material, and we have already had occasion to note instances in which this undoubtedly occurs. It may be that such a precipitation may later involve vacuole formation. This we can at present not decide. It is at least certain that in degenerating cells, vacuoles very frequently appear in the protoplasm. Such vacuolization was described by GALEOTTI '95 for the kidney cells of poisoned salamanders. The most enthusiastic observer of vacuole formation was undoubtedly ALBRECHT. He published numerous papers between 1898 and 1907, papers which are richer in ideas and interpretations than in actual observations. An understanding of ALBRECHT's ideas is perhaps best obtained from his general review written in 1907. ALBRECHT found that when kidney cells were treated with water, numerous vacuoles appeared in the protoplasm. He described the same phenomenon for various other cells, including sea-urchin eggs. ALBRECHT seems to have regarded protoplasm as essentially a mixture of two fluids. Under certain conditions, one fluid might separate from the other just as droplets of phenol separate out from a mixture of phenol and water. Because of this supposed resemblance, ALBRECHT always refers to vacuole formation in protoplasm as "tropfige Entmischung". ALBRECHT apparently never attempted to identify the fluid which

formed the non-aqueous phase in his imaginary system, and he never showed that the vacuoles contained anything but water. In some cells, the vacuoles must surely consist of water, for the volume of the vacuoles is greater than the entire volume of the cell before vacuolization. This, indeed, is true of the sea-urchin egg studied by ALBRECHT. How such a cell could produce a larger volume than its own of non-aqueous fluid, when three-fourths of its original volume was water, is indeed a mystery. It is surprising therefore that the term "tropfige Entmischung" has received as serious consideration as it has.

When cells are studied in tissue culture, degeneration is generally accompanied by the appearance of vacuoles in the cytoplasm. This has been noted by a number of investigators. LEWIS '19 states that "This vacuolization of the cytoplasm is one of the most common modes of cell degeneration and death in tissue-cultures". Other references to vacuolization in tissue cells are the following: LEWIS and LEWIS '15, BURROWS and NEYMAN '17, LEWIS '23 a, b, LEWIS and LEWIS '24, RUMJANTZEW '27, and LUNA '17. However the vacuoles described by LUNA are believed by LEWIS '19 to be mitochondria vesicles rather than degeneration vesicles. An observation of LEWIS and LEWIS '15 may have importance. They find that "in many cells there is often a coincident change in the shape of the mitochondria until in cells which contain many vacuoles within the cytoplasm the mitochondria are no longer in the shape of rods and threads but then appear as small granules". Perhaps the appearance of the vacuoles is associated with a decrease in the amount of the formed elements, and especially the mitochondria of the protoplasm. When vacuoles appear in kidney cells, certain rodlets characteristic of these cells disappear (ALBRECHT '00). And in sea-urchin eggs, as will be pointed out later, vacuole formation is always accompanied either by a disappearance of definite granular elements in the protoplasm, or by a loss of substance from these elements. And the granules which disappear (the pigment granules) are perhaps homologous with mitochondria (see p. 20).

It is in sea-urchin eggs that the process of vacuole formation has received the most attention. ALBRECHT '99 was perhaps the first to notice it in these eggs, and in his papers he often refers to the process in these cells and compares it with similar vacuole formations he observed in other types of living material. But

our knowledge of vacuole formation in sea-urchin eggs is due mostly to LOEB. His attention seems to have been first called to it in 1904. In that year (LOEB '04) he describes what he calls a cytolysis of the sea-urchin egg. The cytolized egg loses its pigment and becomes converted into a pale shadow, at the same time increasing greatly in volume. A similar change had probably been seen by O. and R. HERTWIG '87, for in their paper they refer to the characteristic glassy appearance of eggs treated with a toxic dose of chloroform. LOEB was the first to use the term cytolysis for marine eggs. He seems to have borrowed it from the literature of immunology, where it has had a long history (for a survey of the older immunological literature, see LONDON '02). But LOEB does not use the term in exactly the same sense in which it is used by the immunologists. From its derivation cytolysis means a solution of cells, and in a typical, or perhaps we could say a complete cytolysis, the cells dissolve and disappear. In modern immunological literature, cytolysis denotes such a solution or disappearance of cells (see for example LAUFBERGER '27). In the sea-urchin egg, according to LOEB, cytolysis involves increase in volume, and loss of pigment. Later ('06), he refers to this type of cytolysis as pale cytolysis, in contrast to a dark cytolysis in which the egg does not increase in volume. In this dark cytolysis, the egg breaks up into a mass of small droplets. As a matter of fact, in both types of cytolysis as distinguished by LOEB, there is droplet or vacuole formation throughout the egg. In LOEB's book published in 1913, he shows no droplets or vacuoles in an egg cytolized in distilled water (Fig. 58), but vacuoles are clearly shown in the case of an egg cytolized by salicylic aldehyde (Fig. 51). It is however true that vacuoles do appear in eggs cytolized in distilled water (compare ALBRECHT '99).

It is an unfortunate circumstance that no one has ever studied from a morphological standpoint what LOEB chose to call cytolysis. From many unpublished observations on cytolized eggs, it seems certain that in every case droplets or vacuoles appear in the protoplasm.

It is an open question whether or not it is advisable to retain the term cytolysis as applied to sea-urchin eggs. But it is at least certain that in these eggs there is a reaction in which numerous vacuoles appear in the cytoplasm. This vacuole formation is always accompanied by a diffusion of pigment from the egg into

the surrounding medium, in the case of those eggs which have a visible pigment. In the *Arbacia* egg, the pigment granules may break down completely, or they may merely lose some of their pigment and still remain distinguishable within the egg. In what LOEB called pale cytolysis, the pigment granules completely disappear. In his dark cytolysis, apparently they do not, and in this latter form of reaction, the egg does not undergo a great increase in volume.

In LOEB's studies on what he called cytolysis of sea-urchin eggs, he was influenced by the literature on hemolysis, and especially by the papers of KOEPPE (see KOEPPE '03). KOEPPE had found, and he knows, that hemolysis of red blood cells is caused by water, by heat, by acids, by alkalies, and by fat solvents. LOEB and his student VON KNAFFL-LENZ studied the effect of these various agents on sea-urchin eggs, and it is LOEB's opinion that all of them cause a cytolysis of the cortical layer of the eggs. LOEB's ideas on the subject of this cortical cytolysis were always a little vague, but he was certain that any agent which produced activation or the beginnings of development in sea-urchin eggs, also occasioned a cortical cytolysis. On longer exposure to these parthenogenetic agents, there was a complete cytolysis. From VON KNAFFL-LENZ's work and from LOEB's book ('13), it is clear that water, heat, fat solvents, and also the well known hemolytic agent saponin all cause cytolysis of the sea-urchin egg. According to VON KNAFFL-LENZ '08 fatty acids cause a cytolysis of the sea-urchin egg, but mineral acids have no such action. On the other hand, DRAGONI, VLÈS, and ROSE '23 describe a cytolysis in the presence of mineral acid. The difference in results may be due to a difference in material. From VON KNAFFL-LENZ's account, it is not clear whether alkalies cause cytolysis or not. Certain it is that most of the agents that typically cause hemolysis of red blood cells do actually cause vacuole formation and loss of pigment from sea-urchin eggs.

It should be pointed out that when sea-urchin eggs are left in dishes containing sea-water, they undergo a degeneration process or a cytolysis which also involves a vacuolization and a loss of pigment. It is apparent, therefore, that in sea-urchin eggs there is a reaction which resembles on the one hand the vacuolization phenomena as observed in plant cells and in tissue



culture cells, and on the other hand is related to the well-known hemolysis process of red blood cells.

Just what does this hemolysis consist of? When a red blood cell becomes hemolyzed, pigment escapes from it. This appears to be the essential criterion of hemolysis, although in many instances escape of pigment is accompanied by marked increase in the volume of the red blood cell: it is then said to become a "ghost". NOLF '09 defines hemolysis as a destruction or deterioration of the red blood cells, and he says that when it is complete, the cell completely disappears. Our knowledge of hemolysis is very old. MILNE EDWARDS '58 cites various references to older literature. The hemolysis of red blood cells in distilled water was described by MÜLLER in 1832, and in his book published in 1835, MÜLLER also described other types of hemolysis. In his studies of the physiology of blood, MÜLLER was apparently not very conversant with the older work of HEWSON. He rarely refers to this author, and indeed he was severely criticized by one of his contemporaries for this failure to make proper reference. In a posthumous work of HEWSON 1785 (see p. 14), there is a clear description of hemolysis produced by water. "*Ubi multum aquae additum est, vesicula sensim tenuior magisque pellucida reddetur, et tandem dissolvetur*". When much water is added, the vesicle, that is to say the blood cell, gradually becomes thinner, and more pellucid, and at last dissolves. HEWSON also describes hemolysis in dilute solutions of mineral acids. It is not improbable that there are even older descriptions than those of HEWSON.

Partly because of the importance of the subject, and partly also because it is a relatively simple matter to make observations on the escape of pigment from red blood cells, many authors have studied hemolysis. The literature is now vast. NOLF in 1909 cites well over 200 titles, and he limited himself to the literature of the twenty years preceding. As a matter of fact, practically all of his references are to papers published between 1900 and 1908. Since 1908 there has been no decline in the output of dapers on hemolysis. The bacteriologist, the clinician, the physiologist, even the chemist has attempted to contribute to the solution of the problem of the destruction of the red blood cell. All of this literature does not concern us. The bacteriologist is frequently interested in hemolysis merely because it is a convenient index of the presence or the properties of materials which he is

unable to define chemically. Our primary aim is to show first that certain types of hemolysis involve a species of cell reaction which is found in many cells other than blood cells, and it is our hope that starting from this line of approach we may be able to throw light on some of the mysteries of hemolysis, especially in so far as they are related to the colloid chemistry of the protoplasm of the blood cell.

In general the student of physiology studies only end manifestations. For example he measures the carbon dioxide produced by cells or organisms, or the electric current generated in the course of one vital phenomenon or another, but it is the handicap of the science that in the main the physiologist has little conception of the actual reactions or changes which are responsible for the end results he is able to measure. In no field of cellular physiology is this more true than in the study of hemolysis. Whereas many cells are favorable for microscopic study, the red blood cell is extremely small and it shows little under the microscope. The study of hemolysis has proceeded almost purely along physiological lines. This is a severe handicap. In larger cells, in which reactions similar to hemolysis go on, physiological study can better be combined with morphological study.

The usual type of hemolysis of the red blood cell finds its exact counterpart in the process of cytolysis or vacuole formation as it occurs in the egg of the sea-urchin *Arbacia*. In both sea-urchin eggs and blood cells there is typically an increase in volume accompanied by a loss of pigment. And, as LOEB first pointed out, the reagents which cause hemolysis of blood cells regularly cause what he termed cytolysis of sea-urchin eggs. Distilled water, saponin, bile salts, fat solvents, soaps, on the one hand produce hemolysis, and on the other cause cytolysis of *Arbacia* eggs. It might be thought that there is a difference in the fact that vacuoles appear within the cytoplasm of eggs, whereas one does not ordinarily think of them as being formed during hemolysis. Against this argument, it can be stated first that vacuoles are not always very easy to see even in cells as large as sea-urchin eggs. LOEB often failed to notice them when they were present (compare p. 239). In small blood cells they would be still harder to recognize. Actually, some of the older anatomists in their drawings of hemolyzed blood cells do show structures within the stroma which might very well be interpreted

as vacuoles. In the larger blood cells of Amphibia, vacuoles can more readily be observed. In VON EBNER's revision of KOELLIKER's Handbuch (VON EBNER '02, p. 728), he notes the appearance of vacuoles in such cells following treatment with ammonium chloride. This reagent is a well known hemolytic agent. VON EBNER also figures human blood cells with what he calls vacuole-like spots. BOETTCHER '66 describes the appearance of vesicles and vacuole-like structures in various types of blood cells when they were treated with chloroform vapor, and with distilled water, both typical hemolytic agents. In some cases he apparently mistook vacuoles for nuclei of mammalian red blood cells. Doubtless more thorough search of the literature would reveal other instances in which authors have described vacuole formation following hemolysis. Perhaps it would be simpler to make a few studies of the hemolyzed blood cells of salamanders. It is rather surprising that in recent years these cells have been so little used in studies of hemolysis. But, as a matter of fact, additional evidence is scarcely necessary. From the previous discussion it must be evident that hemolysis of blood cells and what LOEB called cytolysis of egg cells have much in common. It must not be supposed, however, that every reagent which causes a loss of pigment of blood cells will cause a typical cytolysis of the sea-urchin egg. Some reagents cause loss of pigment in *Arbacia* eggs without apparently causing any pronounced vacuolization. This is probably due to the presence of complicating factors.

It is perhaps easier to assume that hemolysis and cytolysis of egg cells are related phenomena, than to assume that cytolysis of egg cells corresponds to the vacuole formations which occur in plant cells. One reason for this is the fact that the evidence that lies at our disposal is very fragmentary. If one considers the action of a reagent like distilled water, it is logical to assume that the vacuoles it produces in plant cells are similar in origin to the vacuoles it produces in protozoa and in sea-urchin eggs. It might however be urged that some of the agents which cause vacuole formation in plant protoplasm have no such effect on animal protoplasm. Thus in the botanical literature mechanical injury has frequently been described as producing small vacuoles in plant protoplasm, and at first sight it might be thought that this did not occur in animal cells, for in recent years at least there has been almost no literature on the subject. But in the

last chapter it was shown that when sea-urchin eggs are torn, many vacuoles appear in the cytoplasm (see Fig. 12), and it may also be remembered that DUJARDIN 1835 described vacuole formation in protozoan protoplasm following injury.

It is interesting to compare the action of the electric current on various types of living substance. In both plant and protozoan protoplasm, the electric current regularly causes vacuole formation. In *Arbacia* eggs it causes cytolysis (LILLIE and CATTELL '25), in red blood cells, as is well known, it causes hemolysis (compare for example ROLLETT '71). It should be noted that in none of these instances was the experimental procedure such that the effects of the electric current could possibly have been due to the production of electrolytic decomposition products.

It might be argued that one of the characteristics of hemolysis and cytolysis is an escape of pigment, and that this has not been observed in plant material. It is true that this phase of the subject has received very little attention on the part of botanists, but BOAS '20, '21 has shown that saponin causes an escape of pigment from some plant cells, and CZAPEK '11 brought together many instances in which fat solvents caused the escape of various substances from plant cells. These it is true were in the cell sap and dissolved. But perhaps even in those cases in which pigment is not seen to be located in visible granules, it is bound to sub-microns, that is to say to ultramicroscopic particles.

Our general conclusion is that in all types of protoplasm there is a definite type of response to such agents as distilled water, fat solvents, the electric current, mechanical injury, etc. This response is typically characterized by the appearance of numerous small vacuoles within the protoplasm. Frequently, though not always, it is accompanied by an increase in the volume of the cell. When pigment is present in the cell, this always escapes.

The reaction that we have described is definite enough to deserve a name. The term "tropfige Entmischung" used by ALBRECHT for certain aspects of the reaction can not very well be used, for it carries with it a highly improbable interpretation of the constitution of the protoplasm. The term cytolysis has certain advantages in spite of the fact that in its original use in the science of immunology it carried various other meanings. But cytolysis, as it is now used, seems to imply destruction, and

it is very doubtful if in all cases of vacuole formation, especially in plant protoplasm, lethal changes are involved. VELTEN '76b clearly states that the vacuoles once formed may disappear again. There can be no question but that a new name is needed, but it will probably be best to wait until the reaction is better understood and more completely and thoroughly described before a distinctive name is given to it.

We are now in a position to inquire into the mechanism of the reaction we have been considering. In no case can the reaction be studied more clearly than in the egg of the sea-urchin. At any rate in no other type of protoplasm aside from blood cells has there been any study of the mechanism of the reaction. In the remaining pages of this chapter, therefore, an attempt will be made to outline some of the factors on which the reaction in the sea-urchin egg depends. In the course of the discussion, occasional reference will be made to the process of hemolysis in blood cells.

In spite of the fact that LOEB many times emphasized the importance of the so-called cytolysis of sea-urchin eggs, he made little effort to explain the mechanism of the process. In pages 188 to 190 of his book ('13), he expresses some vague general ideas regarding the possible nature of cytolysis. Saponin and benzine, he states, dissolve the chorion or jelly of mollusc and annelid eggs. He then cites VON KNAFFL-LENZ's views to the effect that cytolysis is due primarily to the liquefaction of lipoids, and he makes the following quotation from VON KNAFFL-LENZ, "The mechanism of cytolysis consists in the liquefaction of the lipoids, and thereupon the lipid-free protein swells or is dissolved by taking up water."

LOEB thus apparently believes that cytolysis in sea-urchin eggs is primarily a liquefaction of the protoplasm. This it most certainly is not, for viscosity tests indicate beyond the question of a doubt that during cytolysis, the viscosity of the egg protoplasm increases enormously.

MOORE '17a and b, published two papers on the mechanism of cytolysis in sea-urchin eggs. From a study of the temperature coefficient of hot water cytolysis, he voices the opinion that cytolysis is due to a monomolecular chemical reaction. In view of the fact that most chemical reactions in colloidal media behave as monomolecular reactions, this is equivalent to saying that cytolysis is due to a chemical reaction. Very possibly it is, but

unless we know which substances are involved in the reaction, we have scarcely made much progress toward a solution of our problem.

In the next few pages we shall seek to interpret at least certain aspects of what LOEB called cytolysis in sea-urchin eggs. This discussion can not be very satisfactory, for it is based for the most part on experiments now in progress and still unpublished.

As has already been stated, in the *Arbacia* egg, treatment with various reagents is followed by the appearance of numerous vacuoles within the protoplasm, so that the protoplasm eventually becomes a mass of such vacuoles. At the same time, pigment is given off by the eggs. Frequently the egg volume increases, but this volume increase does not always occur, and when it does it is probably rather a cause of the reactions that follow than a result. This will be more clear later.

The first point that should be clearly recognized is that the escape of pigment from *Arbacia* eggs does not depend on the increased permeability of the egg membrane, or rather it is very little dependent on such permeability change. This follows immediately from the fact that the egg membrane or plasma membrane of the *Arbacia* egg is at all times freely permeable to the egg pigment. Anyone who has worked with *Arbacia* eggs knows that the water standing over them is continually colored by pigment escaping from them. Moreover the pigment within the eggs is not for the most part free in the cytoplasm, but is definitely concentrated in easily visible pigment granules which are shown both in Fig. 1 and in Fig. 12. Frequently these pigment granules can be seen to break down, and then the pigment is released both to the rest of the cytoplasm and to the medium surrounding the eggs. As a matter of fact, the reaction we are considering involves a visible change in these pigment granules, and in most instances they disappear completely, with the result that their pigment is dispersed. It is probable that in blood cells too the escape of pigment is not so much due to a change in permeability of the membrane of the blood corpuscle, as it was the fashion to believe around 1900, but rather more to some change within the corpuscle that sets the hemoglobin free. This follows in part from the fact that the hemoglobin can scarcely be contained in solution within the corpuscle, for the corpuscle contains more hemoglobin than it would be possible for it to have in a state of solution. Apparently

among students of hemolysis there is a growing tendency to attribute the retention of hemoglobin within the blood cell more to the binding of the pigment by substances of the cell protoplasm and less to the impermeability of the cell membrane (see for example MOND '25).

Our second point, and this is really the crux of the entire argument, is that the formation of vacuoles throughout the protoplasm of the sea-urchin egg depends on the same reaction that we have called the surface precipitation reaction, and which we have considered in the last chapter. When an *Arbacia* egg is crushed or torn, so that the protoplasm exudes, it may rarely form only a single film or membrane about its free surface. Much more usually, especially if the protoplasm exudes rapidly and there is plenty of calcium in the surrounding medium, the film or membrane formation is not confined to the outer boundary of the emerging droplet. As was pointed out in the last chapter, other films are formed within the main mass of the exuding protoplasm, and these films are in the form of vacuoles. Such vacuoles are clearly shown in Fig. 12. That they are the exact equivalent of the vacuoles which appear in so-called cytolyzed eggs is certain. From eggs which show one or two or a few vacuoles, there is every gradation to those which are filled through their entire volume with vacuoles and are thus, in the common sense of the term, cytolyzed. In order to produce such cytolysis, it is only necessary to crush the eggs very violently.

That the reaction which produces vacuoles is exactly the same as that which causes a membrane to form on the surface of the naked protoplasm follows also from the fact that the same substances play a part in the reaction. Cytolysis as generally described involves a release of pigment from the egg, and this depends of course on a breakdown or at least a partial breakdown of the pigment granules. Thus for vacuole formation in the cell interior, just as for the surface precipitation reaction at the border of an emerging droplet, the pigment granules are essential. Secondly, calcium is also a prerequisite for the appearance of vacuoles. In eggs crushed in the absence of calcium, there is an absence of the surface precipitation reaction, and also a complete absence of vacuole formation in the exuding protoplasm.

We can therefore regard our second point as established. Vacuole formation, as it occurs in the *Arbacia* egg in what has

been called cytolysis, is really an internal surface precipitation reaction.

In the last chapter it was shown that whenever the pigment granules of the *Arbacia* egg were placed in the presence of free calcium in sufficient concentration, a reaction immediately ensued which was the first of a series of reactions comprising the surface precipitation reaction. Apparently the reaction between pigment granule and calcium can occur in the egg interior as well as in the protoplasm pressed out of the egg. If this is the case, then it is only necessary to increase the concentration of free calcium within the cell beyond a certain point in order to produce a vacuole formation throughout the entire egg, or in other words a cytolysis.

If we make the assumption that the calcium within the egg is for the most part bound with some fatty or lipoid substance, then we can offer an explanation which will fit practically all of the known facts. This assumption is certainly a logical one to make. Analyses of protoplasm show a high content of salts, and it is certain that the ions of these salts are in large measure combined with some constituent or constituents of the protoplasm (see p. 25). They can be combined either with proteins or lipoids or both. For the sake of our argument we will assume that the calcium is for the most part combined with lipoid, and furthermore that calcium is set free when the lipoid is dissolved. There is evidence that the surfaces of the protoplasmic granules have a lipoid film (see p. 30). If the calcium were adsorbed at such a film, solution of lipoid would certainly set calcium ions free.

If our reasoning, or our assumption, is correct, the free calcium ion concentration of a cell may be increased in the following ways:

1. By a rupture of the cell membrane.
2. By a decrease in the volume of the cell, as for example when the cell is placed in a hypertonic solution.
3. By long exposure to isotonic solutions of calcium salts.
4. By any treatment which will tend to cause a solution of protoplasmic lipoids. Such treatments include:
  - a) Heat,
  - b) Fat solvents,
  - c) Decrease in the salt content of the cell, i. e. increase in the cell volume.



4c is perhaps not so readily understandable, but it must be remembered that HANSTEEN-CRANNER '19, '22 showed that distilled water caused a solution of protoplasmic lipoids of plant cells. RUNNSTRÖM '23 has moreover presented evidence to show that lipoids in the sea-urchin egg are dissolved when the eggs are placed in hypotonic solutions (Compare also p. 211).

We shall now proceed to show that every one of the methods of treatment outlined in the above scheme does actually cause pigment granule breakdown and vacuole formation in *Arbacia* eggs.

We have already considered the case in which there is a rupture of the cell membrane. When cells are placed in hypertonic solutions, the decrease in volume is of course accompanied by a corresponding increase in the calcium concentration of the interior. In sea-urchin eggs, strong hypertonic solutions cause what LOEB called a dark cytolysis. In this dark cytolysis, much pigment is set free from the pigment granules, and numerous vacuoles or vesicles form within the cytoplasm. It may be remembered that WIESNER '69 also described the formation of vacuoles in yeast cells following treatment with hypertonic solutions.

In the literature, there is no reference to cytolysis following long exposure to calcium solutions. However in unpublished experiments it has frequently been found, as stated in the last chapter, that after long exposure to an isotonic calcium chloride solution, pigment granule breakdown occurs generally throughout the egg. Apparently when eggs are placed in such solutions of calcium chloride, the calcium as it enters the cell is for a time bound or adsorbed so that there is no reaction between it and the pigment granules. But eventually the limit is reached, no more calcium can be bound, or more exactly stated, the percentage of free calcium increases beyond a certain point, and an internal surface precipitation reaction occurs.

Apparently most cytolytic agents act first by causing a solution of fats. VON KNAFFL-LENZ '08 believed that all cytolytic agents acted by dissolving fats, but very obviously neither isotonic calcium chloride solutions, nor hypertonic solutions can be thought of as having a fat solvent action. Heat cytolysis was described by v. KNAFFL-LENZ. Unpublished observations indicate that in this form of cytolysis, at least in the sea-urchin egg, there is not a complete breakdown of pigment granules, but only a loss of pigment from them. VON KNAFFL-LENZ also de-

scribed the action of fat solvents. As a matter of fact, in the action of fat solvents there are really two factors to be considered. Probably the fat solvent acts directly on the lipoids of the cell, at least in most cases, but it must be remembered that the cytolysis in fat solvents is always accompanied or preceded by a great increase in the volume of the cell. This is apparently due to a decrease in the surface tension of the membrane of the cell (see below). The increase in volume produced by fat solvents could in itself cause a solution of lipoids.

There are in general three types of agents which cause increase in volume of sea-urchin eggs. In the first place, hypotonic solutions or distilled water have an effect on the cell readily understandable from an osmotic standpoint. Secondly, substances which have a marked effect on surface tension by lowering the surface tension of the egg membrane, can cause a great increase in the volume of the cell. This phenomenon is not confined to sea-urchin eggs, but occurs in many types of protoplasm. The cell membrane is the seat of various forces, osmotic forces as well as others, and some of these press inward while others press outward. Normally, there is of course equilibrium. The surface tension of the surfaces at the periphery of the cell exerts a force which presses inward. When this force is diminished, the equilibrium is upset, and the volume of the cell increases, either until coagulation sets in, or until the increase in inward pressure due to the distension of the elastic cell membrane compensates for the lowering of surface tension\*.

Substances which lower surface tension certainly cause an increase in volume of free cells generally. But if our conception of the cell membrane is correct, if it is an elastic membrane stretched by the osmotic forces from within, it is obvious that a change in the elastic properties of the cell membrane can also cause a change in the volume of the cell. Many substances cause visible changes in the cell membrane of the *Arbacia* egg and these visible changes are doubtless associated with changes in the elastic properties of the membrane. If the membrane becomes changed so that it takes less force to distend it, then obviously the cell will increase in volume. This is apparently the explanation of the increase in volume which occurs in isotonic solution of sodium chloride and

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\* But compare footnote on p. 203.

various other salts\*. In an isotonic solutions of sodium chloride, the egg remains at its normal size as long as there is a small quantity of sea-water or calcium in the surrounding medium. If this is washed away, then the egg membrane can be seen to swell, and it also becomes sticky. These changes are accompanied by an increase in the volume of the egg, and it goes through a typical process of cytolysis. Such cytolysis in isotonic solutions of sodium chloride and other salts is well known through the observations of R. S. LILLIE, (for references see LILLIE '12). Sodium iodide and sulphocyanate have a much more pronounced effect than sodium chloride. They cause a more pronounced change in the properties of the cell membrane.

Increase in volume, no matter how it is caused, always results in release of pigment and vacuole formation in the sea-urchin egg. The release of pigment is in no sense the result of a change in the permeability of the cell or plasma membrane. Very possibly such a change in permeability may occur, but the escape of pigment is certainly related to visible changes in the pigment granules, for these can actually be seen to give up their pigment.

The release of pigment from *Arbacia* eggs exposed to isotonic solutions of sodium chloride or sodium sulphocyanate or ammonium chloride does not depend directly on the presence of these ions at all, but merely on the fact that in solutions of these salts the eggs increase in volume. Sodium iodide and sodium sulphocyanate have no effect on the release of pigment from the pigment granules. This can be shown by crushing eggs in solutions of sodium iodide or sulphocyanate in the absence of calcium ions. Under these conditions the pigment granules remain perfectly intact and do not lose pigment when they emerge from the egg.

Likewise the effect of saponin is due not to an action on the pigment granules but to some secondary effect. Pigment granules exposed to saponin show no breakdown or release of pigment. On the other hand when entire eggs are treated with saponin, the pigment granules in the interior do break down and disappear. The effect is probably to be attributed to the great increase in volume of the eggs in saponin solutions. A small amount of saponin in the surrounding medium certainly lowers

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\* For other types of explanation, see R. S. LILLIE '11 a, JACOBS '28.

the surface tension of the egg membrane. Whether this is its only effect is not certain. For the purposes of our discussion it is enough to know that the egg undergoes a great increase in volume, and regarding this fact there can be no doubt.

It is believed that the scheme that we have outlined offers an explanation of every type of cytolysis known to occur in the sea-urchin egg. It may not prove to be the correct explanation, but at any rate at present it is the only hypothesis that even attempts to explain the occurrence of pigment granule breakdown and vacuole formation in all cases in which it does occur.

Perhaps this statement should be qualified. LILLIE and CATTELL '25 mention the fact that following treatment with electric currents, some cytolysis was observed in *Arbacia* eggs. If this is the case it is a phenomenon that merits further study, especially in view of the fact that the electric current is known to cause the formation of vacuoles in plant protoplasm, and in protozoa (see p. 235 and p. 236). LILLIE and CATTELL do not state whether the cytolysis they observed was accompanied or preceded by an increase in volume. It is possible that the passage of an electric current may cause the decomposition of those compounds of protoplasm which contain bound electrolytes. This certainly seems plausible, and it is not at all unlikely that calcium ions are set free when an electric current is sent through a cell.

It seems clear that pigment granule breakdown and vacuole formation in the sea-urchin egg, or cytolysis, depend on the occurrence of a surface precipitation reaction in the interior of the cell. We can then conceive of the reaction as occurring in three stages:

1. Calcium is set free in the cell interior.
2. Calcium reacts with the pigment granule or some constituent of it to produce a substance which we have called ovotrombin.
3. Ovotrombin reacts with a substance in the protoplasm, presumably a protein, to cause vacuole formation.

These three stages of the reaction can be studied separately. The various ways in which calcium may be set free in the cell interior have already been indicated. Throughout the discussion, it has been assumed that those agents which dissolve the lipoids of protoplasm set free calcium as a result of this action. As a matter of fact, there is another possible effect of a fat solvent.

Perhaps instead of releasing calcium and thus indirectly causing a breakdown of the pigment granules, it acts directly by dissolving either the pigment itself or some other fat soluble constituent of the pigment granule which then reacts to form ovothrombin. It is possible that the disappearance of pigment granules in eggs exposed to fat solvents depends not so much on a breakdown of the granules as on a loss of pigment and a change in refractive index so that the granules can not easily be recognized even though they may be present.

Stage 2, in which calcium reacts with the pigment granule can very readily be studied separately and such study is now in progress. By taking the pigment granules outside of the egg and watching their behavior toward calcium in the presence of various other substances, much information may be obtained regarding the actual nature of the reaction which occurs between pigment granule and calcium. Some of the results that have already been obtained from this study have been given brief mention in the preceding chapter. In order to arrive at definite and certain conclusions regarding the nature of the reaction which occurs between calcium and pigment granule, further study is necessary. Fortunately the experiments are easy to perform, and it is probably only a question of time before the reaction will at least in some measure be understood.

Stage 3, in which ovothrombin reacts to form vacuoles or precipitation films, has been studied scarcely at all. Further study of this stage is also planned.

In this chapter it has been shown that there is a reaction common to many, and perhaps to all types of protoplasm. In this reaction films or vacuoles typically appear in the protoplasm. Frequently an escape of pigment or other dissolved substances can be noted. In all the types of protoplasm which have been studied, it seems evident that the reaction in question occurs under essentially the same conditions and following treatment with the same reagents. It is therefore plausible to assume that the mechanics of the reaction are fundamentally the same in all types of living substance. Our study has then brought us to the realization that there is a specific type of colloid chemical reaction which occurs in all sorts of living things and which is probably closely related to the life process itself. Study of the reaction in the egg of the sea-urchin *Arbacia* has indicated that in many

respects the reaction is similar to that involved in the coagulation of the blood in higher animals. This is to be deduced from the fact that the reaction is intimately related to the surface precipitation reaction discussed in the previous chapter, and it is thus essentially an internal surface precipitation reaction. No one who has read the chapter on the surface precipitation reaction can very well doubt that this reaction is similar to that which occurs when blood clots. We have thus but another illustration of the general sameness of living things. In spite of a manifold diversity, the underlying principles which govern the behavior of living organisms are everywhere much the same.

In our interpretation of the mechanism of the reaction we have described, we have depended entirely on the facts presented by a study of the egg of the sea-urchin *Arbacia*. Only future study can tell in how far this interpretation will fit the phenomena as they come to be known in other cells. This much at least seems certain. The hemolysis of red blood cells is so essentially similar to the so-called cytolysis of sea-urchin eggs that it can hardly be supposed that the mechanism of the two types of reactions can be very different. In practically every instance, reagents which cause hemolysis also cause cytolysis, and vice versa. In view of this similarity, it is especially satisfactory to note that already in the case of hemolysis, one or two authors have pointed out resemblances between the process of hemolysis and blood clotting. Several substances which prevent blood clotting have been found also to afford protection against hemolysis, (see PICKERING and de SOUZA '23, PICKERING and TAYLOR '25).

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## CHAPTER XV

### CELL DIVISION

In previous chapters we have considered protoplasm more from a static than from a dynamic standpoint. But living substance is continually undergoing changes, changes which in themselves constitute life. In the final two chapters of this book, an attempt will be made to subject certain aspects of protoplasmic activity to colloid chemical analysis.

Unfortunately there is relatively little that can be said about this extremely important phase of the colloid chemistry of protoplasm. Our knowledge is as yet fragmentary, nor can we be certain that all of the fragments rest on the sure basis of fact. Of all the visible, that is to say mechanical processes that go on in living cells, the process of cell division is certainly the most universal. Every living cell is produced by the division of a parent cell. It is thus a characteristic of protoplasm to be able to reproduce itself by division. Because of the importance of cell division, and because too this process has been more carefully studied from a colloid chemical standpoint than some others, we shall begin our discussion of protoplasmic activity by taking up the known facts with regard to the division of cells.

The usual process of cell division, or mitosis, is extremely complicated. As all books on biology and cytology explain, it involves such structures as centrosomes, astral rays, mantle fibers, etc. Mitosis takes place in a series of stages which are described in detail in cytological texts. The description as given in these books is usually based on the appearances in fixed material. In living cells, unless conditions are unusually favorable, not nearly so much can be seen. With certain types of relatively large and transparent cells, most of the structures seen in fixed cells can also be seen in the living. Cytological studies made around 1880 clearly showed the presence of chromosomes in living cells. Astral rays and centrospheres are also sometimes visible.

On the other hand, there is apparently no evidence of any fibers connecting the two centrospheres in living cells, in spite of the fact that such fibers appear very clearly in fixed material.

From a theoretical standpoint, there are a number of interesting problems involved in the division of a cell. In the first place, there is the question of what initiates cell division. Many cells in higher animals never divide. Protozoan cells divide usually only after they have attained a certain volume, but the mere size of the cell is not the only factor involved. The problem of the initiation of cell division is especially interesting because of its close relation to the problem of cancer. This disease primarily involves an uncontrolled division of cells with a resultant increase in the mass of certain tissues.

In the second place, the student of mitosis is interested in the chemical and physical changes which occur when a cell passes through the various stages of the process. An understanding of these changes would open the way to a physico-chemical interpretation of the mechanism of mitosis. At the present time, there is no lack of theories of cell division. Usually it is easier to make a new theory of cell division than to test an old one. Most of the present theories and hypotheses are based on pure speculation. An author postulates a mechanism which might conceivably effect the division of a cell, or he seeks to find in inanimate nature appearances which simulate the structures described by cytologists in dividing cells. There is no field of biological thought that has suffered more from the use of analogies than has the theoretical study of cell division. Even the crudest superficial similarities between mitotic structures and inanimate phenomena have been regarded as having real significance. Concerning the various theories of cell division, we shall have but little to say, and we shall for the most part confine our attention to the actual facts regarding the physical changes which take place during mitosis.

The problem of the initiation of cell division has been attacked from various standpoints, and with various types of material. Some authors have studied the effect of certain chemicals on the division rate of Protozoa. Thus for example, SPEK '20 showed that the lithium ion had a specific effect in initiating or at least hastening cell division in paramecium. Some authors have also claimed that extract of the thyroid gland had an accelerating effect on cell division in paramecium, but this has subsequently



been denied. In working with an organism like paramecium it is hard to distinguish between an effect on the animal itself and an effect on its food. An interesting type of work is that of HABERLANDT (see HABERLANDT '21, also p. 254). This author found that various types of plant cells were stimulated to divide when they were placed in contact with injured or dying tissue. The effective substances given off by the injured or dying cells HABERLANDT calls "wound hormones". In recent years much interest has been aroused by the experiments of GURWITSCH and his pupils, (see GURWITSCH '26). GURWITSCH claimed that dividing cells give off radiations which induce divisions in other cells. The radiations were thought to pass through quartz but not glass, and GURWITSCH believed them to be composed of ultraviolet rays. Unfortunately GURWITSCH never demonstrated that dividing cells gave off ultraviolet rays. He says that such rays are strongly absorbed by gelatin and can not be photographed. But the books on spectroscopy give directions for preparing photographic emulsions relatively free from gelatin and sensitive enough to detect ultraviolet rays of even shorter wave length than those which pass through quartz. Since the above was written VON GUTTENBERG '28 has published the results of observations made with such emulsions sensitive to ultraviolet rays. There is no evidence of any emission from root tips. Moreover, VON GUTTENBERG has rather convincingly shown that GURWITSCH's striking results are most probably the result of uncritical experimentation. See also SCHWARZ '28.

Because of the great interest in cell division, there have been various other types of work besides those mentioned. Many references to the literature may be found in the extensive review of PRÁT and MALKOVSKÝ '27. For a review of the more morphological literature, see also WASSERMANN '26.

Much of the work on mitosis has been concerned with the investigation of factors which might possibly operate during the life of the cell and cause it to divide. But from the standpoint of the mechanism of mitosis, we are concerned not so much with these factors, but rather with all the factors normal or otherwise which can incite a cell to divide. That there are many and varied types of treatment which stimulate to cell division is clearly shown by the work on artificial parthenogenesis. This work has the advantage that it is not concerned with an increase in the division rate of the cell but with an initiation of cell division. An egg

cell does not divide at all unless it is fertilized. And yet when certain types of eggs are treated with the proper reagents, they can readily be made to divide. The initiation of cell division in marine ova was first discovered by O. and R. HERTWIG '87. Following their experiments and the experiments of MORGAN '99, the subject was studied in great detail by LOEB, DELAGE, R. S. LILLIE, and many others. These workers were for the most part interested in the theory of fertilization, they hoped in so far as possible to obtain a normal development of the treated eggs. But in spite of the fact that the students of artificial parthenogenesis did not consider the theoretical study of cell division as their main aim, the results in this field can nevertheless be used in the attempt to explain the mechanism of mitosis. When an unfertilized egg is treated with reagents which incite it to develop, the first important step in the development is the division or segmentation of the egg. Any reagent which can initiate a fairly normal division is almost certain to start the eggs on a development which will proceed for a considerable time; for once the process of development is begun, the successive steps follow automatically.

In the early work on artificial parthenogenesis, it was often felt that certain specific substances were necessary for the initiation of cell division and subsequent development. Later experiments, however, disclosed an ever increasing range of treatments which were effective in causing segmentation in one type of egg or another.

When an egg is fertilized, there is usually some sort of change in the egg cortex, and this cortical change is then followed by changes in the egg interior. So too in artificial fertilization, or rather artificial parthenogenesis, both cortical and internal changes occur. This was first emphasized by LOEB, (see LOEB '13). His favorite method of inducing artificial parthenogenesis involved two successive treatments. Often the reagents which produce an appropriate cortical change have little or no effect in initiating segmentation. According to LOEB, however, they constitute the real initiating factor; the second treatment is in the main corrective. This general idea has been severely criticized by JUST '22, who points out that treatment of sea-urchin eggs with hypertonic solutions alone is as effective as any combination of successive treatments. It should nevertheless be clearly understood

that in every type of egg studied, normal segmentation can only be successfully induced if it is preceded by some type of cortical change. Probably this cortical change has, among other things, the effect of removing the constraint due to the presence of a rigid membrane (HEILBRUNN '15a, '20c). Most eggs before fertilization are surrounded by a stiff membrane, and we can hardly conceive of their being able to constrict as long as this membrane is present. The removal of this obstacle occurs most frequently either as a result of a swelling or softening of the membrane, or following a lifting off of the membrane. In the egg of the clam *Cumingia*, the membrane becomes swollen. In sea-urchin eggs, the membrane is normally elevated, but with certain reagents and also under certain conditions of insemination, it too becomes swollen.

The cortical change can therefore be thought of as removing a block to segmentation. Concerning the nature of the factor which really produces an initiation of segmentation, there have been many theories proposed. In LOEB's later work he held that initiation of development was due to a superficial cytolysis and an increase of oxidations. He thought of the superficial cytolysis as involving the swelling of a colloid at or near the surface of the egg. This colloidal swelling produced membrane elevation if the egg was left in the proper solution for just the right length of time. Longer treatment involved swelling of colloids throughout the entire egg, and the whole cell became cytolized. LOEB believed further that cytolysis in itself produced an increase in the rate of oxidation, and this he also regarded as an important factor. From the preceding chapter it is evident that cytolysis can not be thought of as simply involving a swelling of protoplasmic colloids. Instead of producing a liquefaction, as LOEB supposed, it really causes a gelation or coagulation of the egg contents. Nor does cytolysis of itself necessarily involve an increase in oxidations, in spite of the fact that this was claimed by LOEB and WASTENEYS '13b. In their measurements of the oxidation rate of cytolyzing eggs, LOEB and WASTENEYS determined oxygen by an iodine titration method, and their determinations were rather a measure of the amount of iodine adsorbed by the organic matter so abundantly given off by cytolyzing eggs, than a measure of the amount of oxygen consumed (see HEILBRUNN '15b).

In spite of these criticisms that can be launched against LOEB's ideas, it is not at all unlikely that there is a certain amount of truth in the idea that cytolysis plays a real rôle in the initiation of segmentation. LOEB showed that very frequently too long an exposure to parthenogenetic agents produced cytolysis in the sea-urchin egg. We shall see later that there is some reason for believing that the parthenogenetic agents produce an incipient cytolysis in the egg, and this is not so very different from a superficial cytolysis.

Concerning the permeability theory of artificial parthenogenesis, we shall have little to say, as the discussion would lead too far afield. The chief advocate of the theory has been R. S. LILLIE. His papers are cited in various books on experimental embryology (see for example F. R. LILLIE '19). Perhaps initiation of cell division does involve an increase in the permeability of the cell membrane, but certainly the evidence that has been gathered is far from satisfactory. In recent years, fortunately, there has been a growing tendency to regard the permeability data critically. Most of the older observations which seemed to show increased permeability in eggs stimulated to divide would hardly stand the test of this modern criticism.

Various authors have suggested that artificial parthenogenesis or the initiation of segmentation might be due to a coagulation of the protoplasm. LOEB seemed to favor the idea in 1900, but he soon abandoned this view and became a violent antagonist of it. DELAGE for many years maintained that artificial parthenogenesis was the result of a coagulation followed by a liquefaction, and he rather unwisely considered membrane elevation as one evidence of such a coagulation. However, in his book (DELAGE and GOLDSMITH '13), he became an adherent of the LILLIE theory of increased permeability. Largely on theoretical grounds, the coagulation theory was vigorously proposed by FISCHER and OSTWALD '05. They argued that all parthenogenetic agents are of such a nature as to produce coagulation of the protoplasm. At the time of FISCHER and OSTWALD's paper, there was no knowledge of which agents would or would not cause coagulation of the protoplasm, so that their arguments were not very convincing. Later OSTWALD '07 retreated from his original position and admitted that coagulation in artificial parthenogenesis might be only secondarily produced as a result of increased oxidation.

The first experimental evidence either for or against the coagulation theory was that of HEILBRUNN '15a. He studied the initiation of cell division in the egg of the sea-urchin *Arbacia*. Using the centrifuge method, he showed conclusively that all of the agents which incited the egg to divide, actually did cause a coagulation or gelation of the protoplasm. Following treatment with saponin, with chloroform, with toluol, with isotonic sodium chloride solution, or with 1 % egg albumin in sea-water, there was a very pronounced increase in the viscosity of the protoplasm of the eggs. In general in the *Arbacia* egg, as HEILBRUNN points out, successful artificial parthenogenesis is only possible if the egg is treated with hypertonic solutions. When eggs are treated with distilled water or with fat solvents, it is but a small percentage of the eggs that shows any sign of division, and this division is always abnormal.

Apparently only in the case of the sea-urchin egg is treatment with hypertonic solutions an efficient method of inducing segmentation. Other types of eggs do not respond to this treatment. In the case of the annelid *Nereis*, JUST '15 found that treatment with temperatures of 33 to 35° C. caused a high percentage of the eggs to divide, and the division was generally normal. When *Nereis* eggs are exposed to temperatures of 33—35°, the first change is an extrusion of jelly from the cortex. At the same time the large nucleus or germinal vesicle breaks down. This breakdown of the nucleus is accompanied or followed by a liquefaction of the protoplasm. If the eggs are exposed to the warmth just long enough to produce this breakdown of the nucleus and the subsequent liquefaction of the protoplasm, no segmentation occurs. But if the eggs are exposed a little longer to the warmth, then the protoplasm begins to show a coagulative change, which is very clearly demonstrable by the centrifuge method (HEILBRUNN '25b). The coagulative change was found to occur after about 19 minutes exposure to a temperature of 33°, and after about 13 minutes exposure to a temperature of 34.9°. If the eggs are to be stimulated to divide, they must be allowed to remain in the warmth until this coagulative change has occurred.

Similar results were obtained with the egg of the mollusc *Cumingia*. This egg may also be stimulated to divide by heat treatment. But again, only exposures long enough to produce coagulative changes in the protoplasm are effective. Finally

in the egg of the sea-urchin *Arbacia*, in which exposure to heat produces only a small percentage of divisions, the heat must cause a coagulation of the protoplasm if it is to have any effect.

It is evident that the experimental evidence, as far as it goes, is all in favor of the view that stimulation to cell division in the case of the eggs of marine invertebrates is always accompanied or rather preceded by coagulative changes within the protoplasm.

Apparently in plant cells, too, stimulation to division is dependent on a coagulative change in the protoplasm. In various types of plant tissues, injury or wounding causes many cells to divide which would otherwise have remained in the resting state. It is a well known fact that when plant tissue is cut or otherwise injured, there is a stimulus to growth or proliferation, which may manifest itself in the formation of a callus, or may result merely in the regeneration of missing elements. This knowledge is very old, and must go back to the ancients. Unfortunately, the standard books on plant physiology, usually so full of pertinent information, tell very little about this process. The books on regeneration are more concerned with the problem of form than with the stimulus to growth. A useful paper which may be consulted is that of MASSART '98. This has references to some of the older literature. In recent years the subject has been studied experimentally by HABERLANDT '13, '21. This author has brought forward evidence to show that the stimulus to division is produced by the formation of special chemical substances, which he calls hormones. For the present, this point need not concern us. What HABERLANDT has clearly shown is that when various types of plant tissue are cut, a stimulus to division passes to the cells even several layers distant from the actual cut. Now it seems certain that in these cells the protoplasm becomes coagulated or rather undergoes a great increase in viscosity. This has been shown by various authors (see p. 128), but especially by BUENNING '26 a, b. It will be remembered that this author in three types of plant material found viscosity increases of about 400 to 600 percent in the protoplasm of cells near a cut surface. The viscosity increase was reversible. It is interesting to note that the magnitude of the viscosity increase is very similar to the magnitude of the viscosity increase which precedes mitosis in some types of marine egg material (see below). BUENNING'S

work clearly demonstrates that the initiation of cell division which occurs in plant material in the neighborhood of a cut or wound is accompanied or preceded by a gelation of the protoplasm. Perhaps in animal tissue too the stimulus to division which follows a cut or a wound, and which results in healing or regeneration or sometimes in abnormal growth, may depend on the production of a coagulative change or a gelation in the protoplasm of the cells which divide. But in animal tissue there is no direct evidence in support of this point, and in most animals the problem is complicated by the migration of cells to the neighborhood of the cut.

Cutting or wounding is not the only stimulus which incites plant cells to division. HABERLANDT '19a, b and '20, describes cell division following treatment with hypertonic solutions in the hair cells of *Coleus Rehnellianus*, in the cells of the scales of the onion, and in cells of *Elodea*. These cells therefore behave like sea-urchin eggs. There is not much question but that in the plant cells too the hypertonic solutions cause a marked increase in protoplasmic viscosity.

Both in various types of plant tissue and in the eggs of marine invertebrates, those agents which stimulate cells to divide regularly cause a coagulative change or a gelation in the protoplasm. Concerning the possible nature of this change, we shall have more to say later. That it is directly related to the division of the cell is indicated by the experiments of HEILBRUNN '20a. In these experiments it was shown that if the protoplasm of sea-urchin eggs was kept fluid, no mitosis ever occurred. There is an abundance of evidence (see chap. 11), that fat solvents generally, if they are present in low concentration, have a liquefying action on protoplasm. Following fertilization, *Arbacia* eggs normally complete their first division after about 50 minutes, and the division is preceded by a coagulation or gelation of the protoplasm. If, however, *Arbacia* eggs after fertilization are placed in solutions containing fat solvents in the proper concentration, the gelation which precedes cell division is prevented, and under these conditions the egg always fails to divide, although it is not permanently injured.

We are thus led to conclude first that stimulation to cell division, no matter how produced, always involves a gelation, that is to say a large increase in protoplasmic viscosity; and

second that prevention of this gelation prevents the division of the cell. This is strong evidence that the gelation is intimately related to the division process.

To know that a gelation, or a sharp increase in protoplasmic viscosity, precedes cell division is important, but it is still more important to inquire into the nature of the gelation and to discover if possible the mechanism which underlies it. Before going into this more theoretical phase of the subject, we shall consider the evidence concerning the viscosity changes during the whole course of the mitotic division. Here we are on more certain ground.

REINKE '95 upon subjecting unfertilized and fertilized sea-urchin eggs to pressure, noted that the protoplasm flowed less readily from the fertilized eggs. Similar observations were made by ALBRECHT '98. The differences noted by these authors were in part due to changes in viscosity, but they were also quite as much due to differences in the boundary conditions of the emerging protoplasm, especially in view of the fact that the fertilized sea-urchin egg is surrounded by very different membranes from the unfertilized egg.

KITE '13 and KITE and CHAMBERS '12 dissected various types of dividing cells with microdissection needles. Apparently they noticed no changes in the viscosity of the protoplasm, although they must have been on the lookout for such changes, for KITE makes some vague general statements as to viscosity changes which might theoretically be supposed to occur. HEILBRUNN '15 centrifuged fertilized and unfertilized *Arbacia* eggs simultaneously and found that the granules moved much more readily through the egg before fertilization than after. He concluded that the sperm caused a gelation of the protoplasm of the egg, and that this gelation was directly related to the formation of the mitotic spindle. HEILBRUNN's experiments were not very numerous, and moreover he did not guard against a disturbing factor which tends to obscure the results in the fertilized sea-urchin egg. In *Arbacia*, as soon as the egg is fertilized the pigment granules migrate closer and closer to the egg periphery. They assume a position near the outer border of the cell. This is a fact long known, but not very often mentioned in the literature. The migration of the pigment granules is illustrated in a figure of WILSON '26.



Much more accurate data regarding the viscosity increase in the protoplasm of the sea-urchin egg following fertilization and during the various stages of mitosis, were obtained by HEILBRUNN '17, '20a. He centrifuged the eggs at short intervals during the whole period between fertilization and cleavage, and his results show clearly the viscosity changes which occur in the main mass of the protoplasm. As has just been mentioned, following fertilization most of the pigment granules migrate to the cortex, and there they are not affected by centrifugal force, but remain firmly lodged when the egg is centrifuged. This failure of the pigment granules to move may be due either to their position close to the outer membrane of the cell, or to the fact that there is some sort of a gelation or a coagulation which occurs in the cortex of the egg following fertilization. HYMAN '23, on the basis of cutting experiments with fine steel needles, is of the opinion that there is such a change, but as has been pointed out previously (see p. 41), no definite conclusions can be drawn from this sort of evidence. In the main mass of the protoplasm, there is a very pronounced viscosity increase soon after fertilization. This reaches a height just before the appearance of the mitotic spindle. As soon as the spindle has been formed, the viscosity decreases again, finally rising just before division (HEILBRUNN '21).

In 1917, CHAMBERS again took up the microdissection of dividing cells, and in his preliminary note ('17a), he describes changes in viscosity during mitosis. According to this account, the astral rays are fluid, and the regions between them solid. CHAMBERS states "A periodic reversal of the sol to the gel state and vice versa has been demonstrated in the cell protoplasm during division. The steps taken may be divided into the following series: a. When the monaster is fully formed the greater part of the cell is a gel. b. As the centrospheric fluid collects on the two poles of the nucleus the cytoplasm reverses to a sol state and the monastral radiations fade out. c. The formation of radiations about the centrospheres, one on each side of the nucleus, produces the diaster, and is accompanied by a return to the gel state. d. A return to the sol state later takes place in the equator of the cell. e. The nuclear spindle now divides followed by a constriction around the middle of the cell which continues until the cell is cut into two. f. The reversal of the gel to the sol

state usually starts in the equator of the cell and spreads to the poles." Apparently in this account, CHAMBERS believes that the protoplasm is first a sol, then a gel, then a sol again, then a gel, and then a sol again. As to the times when these changes occur, he supplies no precise data.

CHAMBERS '17b gives very little additional information concerning viscosity changes in the mass of the protoplasm. Following the entrance of the sperm, an aster is formed. That the cytoplasm around this "has been rendered fairly solid is shown by the fact that the aster at this stage may be pushed and rolled in the surrounding liquid cytoplasm". Certain other viscosity changes are inferred, but no definite times are given. "After a space of time (about ten minutes) a change takes place in the cytoplasm about the equator of the nuclear body which appears to be a reversal to the sol state. I was not able to convince myself on this point by the use of the needle." Apparently this is the change referred to under d in the quotation from the 1917a paper. But now CHAMBERS is not certain of a change in viscosity.

CHAMBERS '19 reports further observations on the changes in viscosity during mitosis. A practically complete statement of these observations is given in the following quotations:

„Immediately after fertilization the cytoplasmic granules readily flow by the moving needle. (This is taken to indicate low viscosity). — — When the sperm aster is at its full development, the highly viscous state of the protoplasm is detected by the needle — — — — This condition is at its height 10 to 15 minutes after fertilization. Fifteen to 20 minutes after fertilization, the radiations of the aster begin to fade from view, with a reversal in the cytoplasm of the semi-fluid to a more fluid state. — — — — Toward the end of this stage, which lasts for 20 to 30 minutes. — — — — Shortly before cleavage, about 40 to 50 minutes after fertilization, an increase in firmness sets in — — — — The time of appearance of the amphiaster until completion of cleavage lasts from 10 to 15 minutes. The increased viscosity of the egg during this amphiaster stage. — — — — After the completion of the cleavage process, there are indications that the firmness of the cytoplasm persists". In 1919, CHAMBERS believed in a succession of sol, gel, sol, gel states. But in 1921 (CHAMBERS '21) he has again returned to his 1917 opinion that just before division there is a liquefaction of the protoplasm.

In order to understand CHAMBERS' views, an attempt was made to record his 1919 observations in rough graphic form. No exact reproduction is of course possible, for the time intervals given by CHAMBERS would be different for every temperature, and CHAMBERS makes no mention either of temperature or of the total time between fertilization and cleavage in any given experiment. In Fig. 14, the dotted line represents CHAMBERS' 1919 results, the C's indicating the points at which observations were recorded. On the same graph are plotted the results of

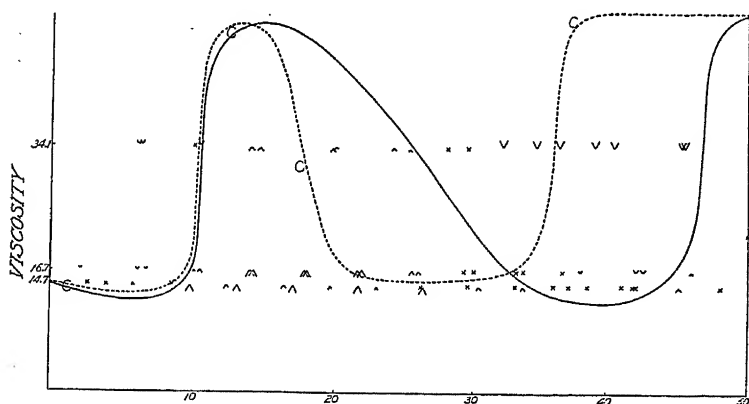


Fig. 14. Viscosity changes during mitosis in the sea-urchin egg. The unbroken line is plotted from the centrifuge data (HEILBRUNN), the dotted line is a rough estimate based on the microdissection experiments of CHAMBERS

HEILBRUNN's centrifuge tests, mostly from the 1920a data. These centrifuge data refer to the *Arbacia* egg. In CHAMBERS' experiments, it is not always clear which type of egg he is referring to. The abscissae of the graph are in minutes, and in every case, for the centrifuge data, the time has been corrected to fit in with a standard time between fertilization and cleavage of fifty minutes\*. For example, if the actual time between fertilization and cleavage was found to be sixty minutes instead of fifty, each time interval

\* The time of cleavage was taken as the time when fifty percent of the eggs showed cleavage furrows.

was multiplied by five-sixths. The ordinates represent viscosity values. The height of the curve is more or less speculative, but is based to some extent on unpublished experiments. In the figure, the V's indicate viscosity values less than the value for the ordinate at that point, the inverted V's indicate viscosity values greater than the value for the ordinate at that point. The length of the V's is also significant, a large V indicating a much lower viscosity value.

It is to be noted that both CHAMBERS and HEILBRUNN find the same cycle of viscosity changes. But the two curves are far from identical. The very stage in which HEILBRUNN records the lowest viscosity is for CHAMBERS a stage of highest viscosity. CHAMBERS thinks that at the time of the amphiaser, the viscosity of the protoplasm is at its peak. Centrifuge tests indicate that when the spindle is fully formed, the viscosity is low. The point is an important one, for on the basis of his belief CHAMBERS has expressed a general theoretical interpretation of the mechanics of mitosis. The microdissection estimates of SEIFRIZ ('20) agree with the centrifuge tests in regarding the amphiaser stage as one of low viscosity.

It might be worth mentioning that if centrifuge tests are made at relatively high temperatures, for example at 27° or 28°, results more like those of CHAMBERS can be obtained. Under these abnormal conditions, the liquefaction which normally follows the appearance of the spindle becomes less and less pronounced (at higher and higher temperatures), and eventually, at temperatures just below the death temperature, no such liquefaction occurs in the injured cells. Perhaps the effect of higher temperatures may be a factor in explaining CHAMBERS' results, for in his earlier experiments he was apparently rather careless about guarding against heating effects (see p. 207). But it is doubtful whether it is worth our while to attempt to explain the divergent findings of a method of viscosity determination which up to the present has never given dependable results. As the reader of this book must realize, in every instance in which the microdissection method has been used as a means of testing viscosity, it has failed to yield results consistent with those obtained by various other methods, all of them superior to it in accuracy, (see chap. 4 for a discussion of the principles of viscosity measurement).

So far we have considered only the egg of the sea-urchin. In the *Cumingia* egg, the viscosity changes during cell division have been studied in much more quantitative fashion. This egg is in the metaphase of the first polar body division when it is shed into the sea-water. At this time the egg protoplasm is highly fluid, and only a few turns of the handle of a hand centrifuge are sufficient to cause a complete stratification of the granules of the protoplasm. (For a figure of the centrifuged *Cumingia* egg, see p. 45). The protoplasmic viscosity of the *Cumingia* egg

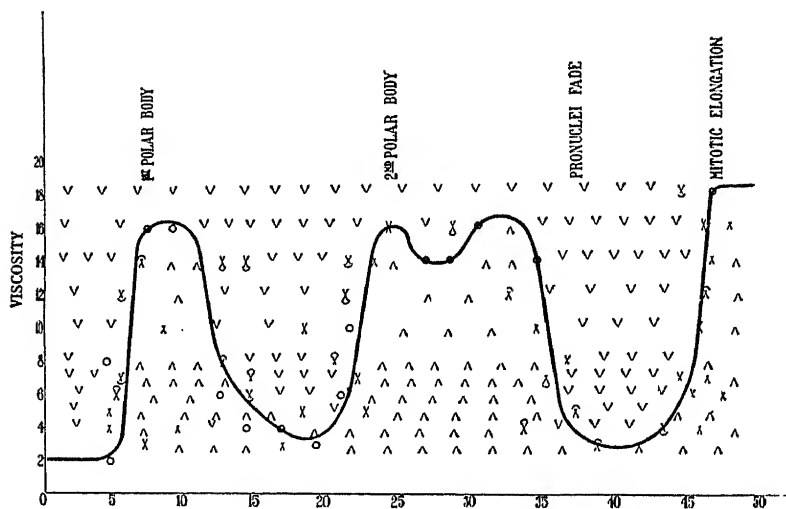


Fig. 15. Viscosity changes in the protoplasm of *Cumingia* eggs during the interval between fertilization and the first cleavage (HEILBRUNN '21)

increases just before the completion of the first maturation division, drops again as the second polar spindle develops, rises as the second polar body is given off, to decrease again with the appearance of the amphiaster of the cleavage spindle. The complete curve of the viscosity changes in the *Cumingia* egg is given in Fig. 15, which is taken from HEILBRUNN '21. In this graph, the X axis represents time in minutes after fertilization, the total time between fertilization and the moment when fifty percent of the eggs segmented being taken as fifty minutes. This was regarded as a standard time interval, and observations made

on eggs which required a longer or shorter time for the period between fertilization and cleavage were plotted by making a correction for the time. The ordinates in Fig. 15 are arbitrary viscosity units and represent seconds of centrifugal turning necessary to cause easily visible migration of the granules of the egg. As a matter of fact, these arbitrary viscosity units happen to be in rather close agreement with absolute viscosity values in terms of water. Each of the symbols of the figure represents a centrifuge test. The V's and inverted V's have the same significance as in Fig. 14. Inspection of the curve shows that the viscosity undergoes an increase of at least five or six hundred percent and then decreases again to approximately its original value.

The viscosity changes in the *Cumingia* egg are more pronounced than those of the *Arbacia* egg. In the egg of the annelid *Nereis*, there is even less change in the viscosity of the protoplasm during mitosis. When the *Nereis* egg is shed into the sea-water, it has a large germinal vesicle. The viscosity of the protoplasm at this stage is very high. At any rate the granules do not move through the protoplasm when the egg is centrifuged. Soon after fertilization the nucleus or germinal vesicle breaks down, and there is a marked decrease in the viscosity of the protoplasm. This is followed by three successive cycles of viscosity change. At a temperature of about 24°, the viscosity rose to maxima at 20, 35, and 47 minutes after insemination, and these times probably correspond to the times when the two polar body spindles and the cleavage spindle were forming. In this connection it should be noted that the telophase of one division is really the prophase of the next, so that when the viscosity rises during the telophase of one division it remains high during the prophase of the next division. At 65 minutes after fertilization, there is another period of high protoplasmic viscosity, and this is followed in a few minutes by the segmentation of the egg. (Fifty percent of the eggs show cleavage furrows at 69 minutes after insemination).

The changes in the viscosity of the *Nereis* egg are not very great and are not easy to follow. It is interesting therefore to note that FORBES and THACHER '25, in the course of other observations, reported an increase in the protoplasmic viscosity of the *Nereis* egg 20 minutes after insemination. Thus, even in the *Nereis* egg in which the viscosity change is relatively slight,

it has been possible to confirm HEILBRUNN's observations. It is much easier to confirm them for the sea-urchin or *Cumingia* egg. Many observers at Woods Hole have noted the cycle of viscosity change in the egg of *Arbacia*. RUNNSTRÖM '24 has also reported confirmation for a European species of sea-urchin. It is an advantage of the centrifuge method that its findings are sufficiently objective so that they can be repeated.

From RUPPERT's brief statements (RUPPERT '24), it seems safe to conclude that in the *Ascaris* egg there is also a decrease in viscosity following the appearance of the mitotic spindle. RUPPERT used the centrifuge method, but his main interest was in the effect of ultraviolet rays rather than in the viscosity changes which occur during cell division.

In *Arbacia*, *Cumingia*, and *Nereis* eggs, there is a high viscosity of the protoplasm in the prophase before the completion of the spindle, then as soon as the spindle is fully formed, the main mass of the protoplasm becomes more fluid again. Division of the cell is preceded by a sharp increase in viscosity. This viscosity increase occurs in the moment or two before the appearance of the furrow, just at the stage when the cell elongates preparatory to division. In Fig. 15, this elongation is referred to as "mitotic elongation", it is what German authors call "karyokinetische Streckung".

If the main mass of the protoplasm is fluid during metaphase and anaphase, it must not be thought that the spindle itself or the astral rays are fluid structures. The fact that the spindle itself is moved as a whole by centrifugal force is evidence enough that it has form, that it is therefore a solid or semi-solid body. Practically all the authors who have used the centrifuge in embryological experiments have described such movements of the spindle. The experiments of CONKLIN '17 are perhaps particularly impressive in this connection. Moreover HEILBRUNN '20a has brought forward evidence to show that the astral rays are firm elastic structures. When *Arbacia* eggs are centrifuged soon after fertilization, the contour of the egg remains smooth or even, and the eggs are generally quite spherical, at least after they have been removed from the centrifuge tubes. But as soon as the mitotic gelation begins, the eggs assume irregular shapes when centrifuged. They do this not only when the main mass of the protoplasm is of high viscosity,

but also in the anaphase when the protoplasm as a whole is fluid. HEILBRUNN concluded that there are "gelatinous strands, — — — which retain their connection to the egg surface and pull upon it when the egg is centrifuged". HEILBRUNN also cites additional evidence to show that the astral radiations exert a pull on the surface of the cell and are thus gelatinous strands. Soon after fertilization, the surface contour of the (untreated) cell is perfectly smooth, but with the first appearance of the amphiaster it becomes crenate, as though tension were being exerted on it from a number of points on the inside. These crenations appear more plainly if the egg is viewed from one pole of the mitotic spindle. They can be seen under the low powers of the microscope, and in view of the fact that the *Arbacia* egg has been so often studied, it is rather surprising that other authors have not commented on their occurrence. If the astral rays pull on the surface membrane of the cell, this is of course an important factor to be considered in any theoretical interpretation of the mechanics of mitosis. The pull of the astral rays would create local differences in the tension of the cell membrane, and these would of course vary with the development of the asters and their connection or lack of connection with each other.

So far we have considered only the viscosity changes in the dividing eggs of marine invertebrates. Many students of experimental embryology have centrifuged the eggs of the frog, but their interest was almost wholly in problems concerning the organization of the egg. From this older literature, ÖDQUIST has attempted to obtain information concerning the viscosity of the egg protoplasm at various stages of mitosis. He cites three papers, GURWITSCH '04, '08, and KONOPACKA '08. From GURWITSCH '08 he rescues the following statement: "Der Zelldurchschnürung, wie auch der Furchenbildung geht eine beträchtliche Verdichtung des entsprechenden Plasmastreifens voran", (see footnote on p. 517 of GURWITSCH '08). Apparently this is the only statement in GURWITSCH's papers that can be taken as indicating viscosity change, and as far as can be judged from reading the context five or six times, it is not based on any certain physical evidence, but rather on some ideas that GURWITSCH has about a meniscus between two types of materials in the centrifuged egg. KONOPACKA's results yield more information, and ÖDQUIST's summary of them is helpful. Apparently the egg protoplasm is less vis-



cous fifteen minutes after fertilization than before fertilization, and apparently it is still less viscous one and a half to two hours after fertilization. Just after the first segmentation, the viscosity is like that of the eggs fifteen minutes after fertilization. Interpretation of viscosity change in the frog egg is complicated by the fact that the egg takes up water after it leaves the oviduct. ÖDQUIST criticizes KONOPACKA's results on the ground that she did not use eggs from the same batch in comparison experiments. ÖDQUIST himself centrifuged eggs 1, 2, and 3 hours after fertilization, just before the first cleavage, just before the second cleavage, etc. His results indicate viscosity differences during the mitotic cycle, and he believes that in general they fit in with HEILBRUNN's results for marine eggs. In view of the fact that the viscosity of protoplasm may rise and fall, all within the space of a few minutes, it seems certain that viscosity tests made at intervals of an hour are not sufficiently frequent. ÖDQUIST centrifuged the eggs at more frequent intervals during the second cleavage. Here his results are more interesting, especially in view of the fact that in this period there are no maturation divisions. He found relatively high protoplasmic viscosity 20 minutes after the first cleavage, and decidedly lower viscosity 20 minutes later (the time between the first and second cleavages was fifty minutes). These results fit in very well with the results shown in Figs. 14 and 15. However immediately after each cleavage, the viscosity in the frog's egg appears to be relatively low. This evidently constitutes a difference between the two types of material. It seems probable that in many cells the successive mitoses are not as closely linked as they are in the eggs of marine invertebrates. In sea-urchin eggs the end of one division is the beginning of the next, and for this reason perhaps there is a viscosity increase at the conclusion of each division. Such a final viscosity increase may be lacking in other cells, and there is good evidence that it is lacking in the case of the frog's egg.

ZIMMERMANN '23 studied the viscosity of the protoplasm in dividing cells of the alga *Sphacelaria fusca*. Normally the protoplasm in the cells of this alga is in the form of lamellae between vacuoles. In order to study the viscosity of the protoplasm during mitosis, ZIMMERMANN seems to have first plasmolyzed the cell, or at any rate to have changed the protoplasm in such a way that it was no longer a foam. Concerning his results,

he says: "Wir können also in der Prophase eine Zunahme der Plasma-Viskosität beobachten, in der Metaphase eine rasche Abnahme und nachher wieder eine allmähliche Steigerung. Die Versuche stimmen überein mit Zentrifugierungsversuchen, die amerikanische Forscher, u. a. HEILBRUNN (1920), an tierischen Zellen unternommen haben und ferner mit meinen Beobachtungen über die Brownsche Bewegung."

ZIMMERMANN'S data do not appear to be very completely given. In one place he refers to Table 5, which apparently was not included in the published paper. But as far as his results go, they are in direct confirmation of the results of HEILBRUNN on marine eggs. CHAMBERS, in his chapter in Cowdry's General Cytology (CHAMBERS '24) is in error when he states emphatically that ZIMMERMANN'S results are in contradiction with those of HEILBRUNN. This error was pointed out to CHAMBERS and it does not appear in a later imprint of the book.

It is evident that in various types of cells marked changes in the viscosity of the protoplasm occur during the process of cell division and these viscosity changes are doubtless associated with changes in the colloidal state of the colloids of the protoplasm. There is also other evidence in support of this view. As long ago as 1882, FLEMMING in his book on the cell, (FLEMMING '82, see for example p. 206), pointed out that during the process of mitosis there are changes in the refractive index of the cytoplasm. He states that during the prophase the refractive index increases. FLEMMING regarded these differences in refractive index as due to changes in the consistency of the protoplasm, and to these changes in consistency he attributes the differences in the staining properties of dividing cells observed both by himself and by VAN BENEDEN in 1875. When fixed with osmic acid, dividing cells are much darker than resting cells, (see especially FLEMMING '91b). In living epithelium cells of the salamander, FLEMMING describes a clear zone in the center of dividing cells, a zone which probably corresponds to the clear area between the astrospheres in dividing sea-urchin eggs. This clear zone FLEMMING regards as more fluid, for he can see Brownian movement in it. But this is hardly a valid criterion, for Brownian movement would always be more vigorous in the presence of fewer granules.

In a study of dividing connective tissue cells, FLEMMING '91a (see p. 261) notes that cells which had become dark in the early

stages of mitosis, became light again in the late anaphase. Finally in a general paper ('91b), FLEMMING again takes up the question of the optical changes of cells during division, urging that these changes are a constant characteristic of dividing cells, and that they must have physiological significance. He notes with regret that practically no mention of these optical changes is made in the many papers on cell division that appeared between 1882 and 1891.

LEVI '17 also describes changes in the refractive index of dividing cells, but his observations are not as complete as those of FLEMMING, and he is apparently unaware of the older literature.

FLEMMING and LEVI estimated the refractive index of the cells they studied. The first attempt at a quantitative measurement is that of VLÈS '21a, b. VLÈS had the ingenious idea of using the sea-urchin egg as a miniature lens, and then from a measure of the focal distance of this lens to calculate the refractive index of the protoplasm. In making his calculations, VLÈS is obliged to neglect the presence of the fertilization membrane of the egg. He says that the presence of this membrane can have only a negligible effect, for it must have a refractive index extremely close to that of sea-water. Actually there are two membranes around the fertilized egg of the sea-urchin, the fertilization membrane and the hyaline layer. Neither of these membranes has a refractive index very close to that of sea-water, for both are clearly and easily visible, and this would not be the case if their refractive indices were very close to that of sea-water. Hence VLÈS' method of calculating the refractive index is not exact. In VLÈS' observations, he finds a decided change in refractive index only at the time when the egg changes in shape just before division. There may be a real change in refractive index at this time, but it is equally possible that the difference which VLÈS noted may have been due all or in part to the inaccuracy of his method of calculation, based as it is on the assumption that the egg is not surrounded by membranes which differ appreciably in optical qualities from the sea-water. If VLÈS' method of calculation is inexact, this inexactitude would only become apparent when the egg changed shape. It is possible to remove the fertilization membrane from fertilized sea-urchin eggs by shaking them vigorously one half minute after they are inseminated. In this way it would be possible to get rid of one of the disturbing membranes, and one

could make somewhat more accurate determinations of refractive index by VLÈS' method. But even if the fertilization membrane were shaken off, the hyaline membrane would still remain.

VLÈS '24 also studied the surface forces of the egg during cell division. By surface forces, he understands all those forces which resist deformation, (see chap. 6, p. 99). If the astral rays are strands which pull on the periphery of the egg, see p. 272, it is obvious that when the amphiaster is fully formed, the surface forces of the egg, that is to say the forces which tend to prevent the egg from flattening, would be relatively great. From his observations VLÈS actually concludes that this is the case. Unfortunately the data he submits are not very satisfactory. The presence of a fertilization membrane would interfere with VLÈS' method of measurement, (for a discussion of this method, see p. 99). In order to prevent the appearance of a fertilization membrane, VLÈS removes the jelly from the eggs. He is apparently under the impression that membrane elevation in the sea-urchin egg depends on the presence of the jelly. This is certainly not true, as has frequently been shown in recent years. To remove the jelly, VLÈS treats the eggs with 3 % KCN. It would have been simpler to shake them or to centrifuge them, and far less injurious. VLÈS' conclusions are based on the study of five eggs. One hour and twenty minutes after insemination is the time at which the surface forces are greatest and the tendency to resist flattening the greatest. VLÈS' actual data for this time show that two of the five eggs did resist distortion more than the average, a third was about average, and a fourth resisted distortion less than the average. The fifth egg VLÈS did not measure exactly at one hour and twenty minutes after insemination, but in view of the fact that this egg reached the diaster stage some time before the others, it is more appropriate to use the measurement made at one hour and ten minutes after insemination. This fifth egg resembled the fourth egg in showing less tendency to resist distortion than the average. It is thus difficult to obtain any certain information from VLÈS' data. Moreover it is not easy to understand how he could have derived any information regarding surface forces of the egg from a study of its eccentricity or flattening during the time the egg was elongating just prior to division. It is not surprising that at this time VLÈS found the egg more eccentric. It might of course have been possible to draw some conclusions if in the eggs mea-

sured, the axis of the spindle had in every case been exactly in the axis of the horizontal microscope, for if one looks at an ellipsoid from one end, it appears spherical. But of the five eggs studied by VLÈS, only one had the axis of its spindle exactly in the axis of the microscope, and even in this case it is hard to see how one could be certain that the two axes were identical.

If VLÈS' experiments are ever repeated, and it is to be hoped that they will be, they should be performed on a hundred eggs instead of five, there should be no treatment with 3 % KCN, and the fertilization membranes should be removed by vigorous shaking thirty seconds after insemination.

In some cells, the protoplasm shows ameboid movements just before the cell divides or during division. This was noted by PERMESCHKO in 1879. More recently it has aroused the interest of the students of tissue culture, (BURROWS '13, LEVI '17, STRANGEWAYS '23, BUCCIANTO '27). STRANGEWAYS finds that in the prophase the protoplasm is quiet, but that in the anaphase it shows very vigorous movement. This is probably an indication of greater fluidity, although the mechanical disturbances caused by actual division may be responsible for the movement. Probably in cells which do not undergo rapid successive divisions, the viscosity of the protoplasm is low at the conclusion of cell division, (compare p. 273).

Many authors have described the rhythmic variations in the susceptibility of dividing cells to various toxic substances. Such rhythmic changes in susceptibility were first noted by LYON '02, '04, and they have been studied by many subsequent workers. Most of these workers interpret them as due to a periodic change in the permeability of the cell during mitosis, but this explanation is scarcely tenable, for there are rhythms of susceptibility not only to the action of chemicals, but also to the action of heat, cold, and light, (for rhythmic susceptibility to light during mitosis, see RUPPERT '24). Apparently the rhythmic susceptibility of dividing cells is to be correlated with the rhythms of colloidal change which are known to occur during mitosis.

In the discussion so far it has been shown first that all the agents which stimulate cells to divide cause a pronounced viscosity increase, that is to say a gelation or coagulation of the protoplasm, and in the second place it has been shown that certain characteristic changes in viscosity occur during the division

of many different types of cells. It has moreover been shown that the presence of these viscosity changes may be correlated with other known facts regarding the behavior of the cell during mitosis. From the mass of evidence that has been presented, it seems safe to conclude that our knowledge of the viscosity changes in the protoplasm during the division of a cell rests on the sure basis of fact.

We know that there is a viscosity increase during the prophase of the mitotic division, and the evidence is good that this increase is closely related to the mechanics of the division process. But as to the causes which underlie the viscosity increase, we are still very much in the dark. If we are really to come to an understanding of the colloid chemistry of protoplasm, we must seek to explain the colloidal changes of the living substance in terms of the colloid chemistry of inanimate materials. Probably this is as yet scarcely possible, but it may be worth while to consider what possible explanations may be offered for the mitotic gelation.

Apparently there is some sort of correlation between the size of the nucleus and the viscosity of the protoplasm. In general the larger the size of the nucleus, the more viscous the protoplasm. Eggs in the germinal vesicle stage always have a very viscous protoplasm. It has already been mentioned that the immature *Nereis* egg has protoplasm of high viscosity. This is also true of *Arbacia* eggs which are immature and still have a germinal vesicle, and as a matter of fact it seems to be generally true of all types of immature eggs. Moreover in stages of mitosis in which the nuclear membrane is broken down, and there is no nucleus as such, the viscosity is always low. But the correlation is not absolute. Thus in the *Cumingia* egg, when the cleavage spindle forms, and the protoplasm changes from a state of high viscosity to a state of low viscosity, this change does not occur just at the stage when the pronuclei break down, but slightly before. Again in the *Cumingia* egg when the second polar body is fully formed, the viscosity of the protoplasm is low and yet at this time the male pronucleus is intact. It is thus not at all certain that there is a direct relation between the size of the nucleus and the viscosity of the protoplasm, although in some instances at least the breakdown of the nucleus does appear to have an effect on the protoplasmic viscosity.

In a series of interesting papers, RUNNSTRÖM (24a, b, c) has attempted to interpret the mitotic gelation which occurs after fertilization in the sea-urchin egg. He believes he can demonstrate by darkfield study that the lipoids of the egg undergo an increase in degree of dispersion. Following this increase in degree of dispersion, RUNNSTRÖM believes that the lipoids unite with the proteins. The following quotation is from the '24c paper:

“Unsre Arbeitshypothese über das Wesen der Plasmaveränderungen bei der Befruchtung kann nun etwas weiter ausgearbeitet werden. Die Lipide befinden sich bei den unbefruchteten Eiern in einem verdichteten Zustande, was die Wasserbewegung bei diesen hemmt. Nach der Befruchtung wird die Dispersität der Lipide erhöht. Diese verbinden sich dabei zum Teil mit den Eiweißstoffen zu einem kolloidalen Komplexen, das andere Eigenschaften wie die einzelnen Bestandteile erhält.”

As a working hypothesis, this is perhaps as good as any. The truth of the matter is that we are still very much in the dark. There are however a number of definite facts that are known regarding the mitotic gelation. These may be summarized as follows:

1. Comparing the eggs of *Cumingia*, *Arbacia*, and *Nereis*, the gelation is more pronounced the smaller the size of the egg.
2. The gelation is prevented and also reversed by ether and other fat solvents.
3. Cold has a similar effect.

In addition it may be worth while to refer to some preliminary experiments, which it is hoped to extend and amplify some time later. Before fertilization the protoplasm of the sea-urchin egg behaves as a true fluid, that is to say the viscosity as determined by the centrifuge method is independent of the amount of centrifugal force (see p. 96). It might be thought that following fertilization it would no longer show this behavior, for we might very well expect that with an increase in viscosity it would show evidence of elasticity. However, in a few tests no difference in viscosity of protoplasm of fertilized eggs could be noted when tests were made at different centrifugal speeds. These tests are not very easy to make, for one must compare the protoplasm of two different sets of eggs at the same number of minutes after fertilization. In the *Cumingia* egg the tests

that have been made also indicate no difference in viscosity values of protoplasm centrifuged at different speeds at comparable times during the mitotic cycle. Thus even during periods of high viscosity the protoplasm of the *Arbacia* and *Cumingia* eggs seems to show the properties of a true fluid. Further evidence of the same sort is provided by the fact that VLÈS was unable to find any appreciable anisotropy in the dividing sea-urchin egg. According to VLÈS, ERRERA also failed to find anisotropy in dividing plant cells. The observed cells were under the pressure of a cover slip and under these conditions one would expect a gel or an elastic fluid to show anisotropy (see chap. 6).

These results seem to indicate that the viscosity increase of the protoplasm during mitosis is not due to the formation of a network or anything of the sort in the protoplasm between the granules. They also do not favor the view that the viscosity increase can be due to any attraction of the granules for each other, as might have been supposed. Almost the only interpretation that is left is to assume that the mitotic gelation or increase of viscosity is due to the precipitation of granular elements within the dispersion medium which lies between the granules.

In order to test this view fertilized *Arbacia* eggs, during the period of high viscosity, were centrifuged for a very long time at a high rate of speed. If the high viscosity is due to the formation of new granules, and if these are moved by centrifugal force, then there ought to be an increase in the volume of the accumulation of granules thrown to one side of the egg by the action of the centrifugal force. No such increase could be noted, and if it occurs it is certainly not great.

It might thus appear that there is no possible interpretation that could fit the facts. But there is one way out of the difficulty. We can assume that the mitotic gelation is caused by the precipitation out of the fluid part of the cytoplasm of granules so small as to be for the most part invisible, and so small also that they would not readily be affected by centrifugal force. At present this is merely a formal explanation, and it would need more supporting evidence before it could be accepted. It is a point of view that can perhaps be built upon. One might assume that the hypothetical granules were lipid and that they were dissolved by fat solvents. One might also assume that the granu-



les had something to do with the formation of the astral rays and spindle. The advantage of hypothetical granules is that one can assume anything about them. But until more actual evidence is forthcoming, we must admit that this hypothesis is too uncertain.

There is another line of approach that is also interesting. Following fertilization in the sea-urchin egg there is a sudden increase in the amount of pigment given off by the eggs. This is rather a well known fact, and it has been used by R. S. LILLIE '11a as evidence for an increase in permeability following fertilization. In the preceding chapter it has been pointed out that escape of pigment does not depend on permeability, but rather on the loss of pigment from the pigment granule. Moreover it was noted in the last chapter that escape of pigment was always associated with a specific type of reaction which when it was complete involved a vacuole formation in the cytoplasm. Is the fact that pigment is given off immediately after fertilization evidence that the reaction which we described in the last chapter is really at the basis of the mitotic gelation? Apparently it can not be so regarded. For in the sea-urchin egg fertilization is accompanied by the lifting off of a membrane. The momentarily naked protoplasm covers itself with a new film, the so-called hyaline layer or hyaline membrane, and this film formation is certainly a form of the surface precipitation reaction and as such probably involves a loss of pigment from the pigment granules. The escape of pigment following fertilization in the *Arbacia* egg thus seems to depend primarily on this reaction and it may have nothing to do with the mitotic gelation. On the other hand, it must be remembered that the occurrence of a surface precipitation reaction at the periphery or cortex of the egg may very well and probably does involve the production of an excess of the substance we have called ovothrombin (see p. 226) Granted the formation of this substance and our problem is perhaps on the road toward a solution.

In this connection it is interesting to note that HEILBRUNN '27 in some preliminary experiments reported evidence of an anti-thrombin-like substance as being present at some stages of the mitotic cycle in the *Arbacia* egg. One could also interpret an experiment of RUNNSTRÖM's in this fashion. RUNNSTRÖM '24a states that when one subjects unfertilized and fertilized eggs

to pressure, there is a decided difference in the appearance of the extraovate. The protoplasm that emerges from the unfertilized egg is full of vesicles (RUNNSTRÖM thinks of these as emerging from the egg, but this is doubtless an error on his part), whereas vesicles only appear some time later in the protoplasm of the fertilized egg (compare Figs. 14a and b of RUNNSTRÖM's paper). The vesicles figured by RUNNSTRÖM are doubtless the equivalent of the vacuoles so often described in the previous chapter, and it is interesting to note that the vacuoles are less rapidly formed in the protoplasm emerging from the fertilized egg. This might be regarded as evidence for the presence of an antithrombin in the fertilized egg, but it must be remembered that the conditions of outflow are different before and after fertilization, and the speedier production of vacuoles or vesicles in the unfertilized egg might very well be due to a greater speed in the emergence of the protoplasm.

More certain evidence that mitosis may be related to the type of reaction considered in the previous chapter is provided by the experiments of LOEB (see LOEB '13). It will be remembered that in LOEB's later studies on artificial parthenogenesis, he took the stand that all agents which caused cytolysis were parthenogenetic agents. It is perhaps doubtful if all cytolytic agents do really cause segmentation in the sea-urchin egg. Many of them are too toxic. But there is a general tendency for a cytolytic agent to cause mitosis, providing that the exposure to which the egg is submitted is just of the right length. Moreover in eggs other than the sea-urchin egg, perhaps the most common method of inducing segmentation is by exposure to heat, and such exposure may very well induce the type of reaction that LOEB called cytolysis. Note too that in vertebrate eggs, or rather in the egg of the frog, the only successful parthenogenetic method is the traumatic method. The eggs are pricked with a needle, and as a result of this injury they begin to divide (see GUYER '07, BATAILLON '11). Injury by the prick of a needle must produce a surface precipitation reaction, and in the last chapter it was shown that this type of reaction is one and the same as the reaction which LOEB called cytolysis. From this line of reasoning we are thus able for the first time to bring together the facts regarding parthenogenesis of invertebrate eggs and our knowledge regarding parthenogenesis of the frog egg. Both types of parthenogenesis

depend on the same type of reaction. We are also able to understand why the introduction of blood serum into a frog's egg is a more effective method of parthenogenesis than the prick of a needle which is not coated with such serum. It has been shown in the two preceding chapters that the surface precipitation reaction is closely related to the reaction involved in the coagulation of blood and that a thrombin-like substance is involved in it. What more natural to suppose than that the thrombin present in the serum of frog blood would favor a surface precipitation reaction in the egg of the same species?

Finally it should be remembered that in plant tissue there is an abundance of experimental evidence that wounding causes an initiation of cell division. It is probable that in this phenomenon a surface precipitation reaction takes place in the cells directly affected by the wound, and that as a result a thrombin-like substance is produced by these cells. This thrombin-like substance would then be responsible for the gelation in the cells near the wound, a gelation which is almost certainly related to the initiation of mitosis in these cells. Hence in this type of experiment also there is evidence that the reaction which we described in the last chapter is responsible for the mitotic gelation.

Against the point of view that we have been developing it might be argued that in normal mitosis there is no evidence of vacuole formation within the cytoplasm\*. This is apparently an important objection, but fortunately it can be met. In some experiments performed during the summer of 1927, it was found that ovothrombin could produce not only surface films and vacuoles, but also long fibrils in the protoplasm of the sea-urchin egg. In these experiments the eggs were crushed in oxalated sea-water. In the oxalated sea-water, it will be remembered, the protoplasm streams out and there is no surface precipitation reaction. If now some ovothrombin is added to the side of the cover slip which rests over the crushed eggs, the protoplasm which has streamed out of the eggs is suddenly seen to form beautiful long strands or filaments. Examined under high power these filaments appear quite hyaline, but along their length here and there

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\* However, in eggs treated with neutral red, M. and M. PARAT '27 describe the appearance of vacuoles during the mitotic cycle. Whether these vacuoles also form in the absence of neutral red is not certain.

protoplasmic granules are attached to them. The filaments or strands behave as thoroughly rigid structures. When they are pushed to one side or another by water currents of the fluid on the slide, they tend to resume their original positions. In a position of rest the filaments are always perfectly straight. From these experiments it is evident that the type of reaction which we have described in detail in chapters 13 and 14 is capable of forming not only vacuoles but also filaments or strands which bear a close resemblance to our usual concept of astral rays or spindle fibers.

There is thus a considerable body of evidence that reactions of the same type as the surface precipitation reaction really play a part in mitosis. So far the evidence is far from conclusive. Much more work needs to be done before we can be in a position to satisfactorily understand the mitotic gelation. Nevertheless it is believed that the point of view here developed has the decided advantage that it offers a means of understanding all known facts regarding the type of agents which can cause initiation of cell division. In spite of the fact that different types of cells are stimulated to divide by very different reagents and treatments, it now seems possible that all these reagents and treatments result in a single type of reaction, a reaction which results in mitotic gelation and subsequent division of the cell.

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## CHAPTER XVI

# PROTOPLASMIC ACTIVITY

In the last chapter, one type of protoplasmic activity was discussed, and it was shown that the division of the cell depended on colloidal changes in the protoplasm. Beyond any question many other types of protoplasmic activity also involve colloidal change. The prevention of colloidal change inhibits the possibility of activity. The protoplasm may be kept either in a constant fluid state, or it may be kept in a relatively stiff and gel-like condition. In either case the normal protoplasmic activity is suspended, and if this prevention of activity, or of some phase of activity, does not result in the death of the protoplasm it is said to involve a condition of anesthesia. From a colloid chemical standpoint, it seems probable that anesthesia is really due to the fact that the protoplasm is kept either very fluid or in a highly viscous state (compare HEILBRUNN '20b).

If the prevention of activity is irreversible, that is to say if the protoplasm is unable to recover, it is by definition dead. In general only those agents which cause an increased viscosity or a coagulation can produce an effect which is irreversible. At any rate no case is known in which an increased fluidity of the protoplasm is irreversible. We are thus led to the conclusion that death is the result of an irreversible coagulation of the protoplasm. This is by no means a new opinion. Indeed many authors have expressed the view that death is due to an irreversible coagulation of the protoplasm.

There are many different types of protoplasmic activity. An ameba projects pseudopodia and moves from place to place, other protozoa travel by means of flagella. In plants there is typically a response to light, to gravity, and to various other factors of the environment. Blue green algae show peculiar movements which apparently depend on secretion phenomena.

There are numerous examples of chemical activity. Gland cells secrete an endless variety of chemical materials. Muscle cells contract and impulses travel along nerves.

But if we examine more closely, if we study carefully the facts at our disposal, it becomes apparent that there are certain phenomena which have much in common. Many types of protoplasm are thrown into sudden activity, or at least react quickly, to various agents such as an electric current or mechanical pressure, and the response wherever it is found follows the same general laws. It is hardly our purpose to enter a general discussion of the facts regarding irritability. These facts are too well known and there are too many of them. If we embarked into a complete discussion, we might very well lose track of one or two points it is our desire to bring forward.

We shall endeavor to state the most important points as briefly as possible. If in this discussion we refer to facts that are widely known, this need not weaken the force of the argument.

In the first place, if we are to speak of protoplasmic activity, it might be well to consider what is meant by the resting state. For the purposes of the present argument, let us define the resting state as the state in which the protoplasm normally continues for long periods. This certainly is not an ample definition for all protoplasm, or even for any type of protoplasm under all conditions. But for us in this chapter it will prove sufficient. For the purposes of our discussion, it will be necessary to consider streaming protoplasm as in a state of rest, in spite of the fact that it is continually in motion. This is certainly a contradiction in terms, but the trouble lies only in the words, the concept is clear enough. The normal state for some protoplasm is a state of slow motion. It remains in this state for long periods, and as far as is known, in many cases at least, no sudden or special changes in the environment exterior to the cell are necessary to maintain this slow motion.

Consider now the effect of mechanical pressure or of an electric current. Immediately, the motion of the streaming protoplasm is stopped (see p. 125 and p. 132). Moreover the cessation of movement is not limited to the cell immediately treated, or so to say stimulated; cessation of movement travels as a wave from cell to cell. Something has happened both to the protoplasm of the cell immediately affected by the physical agent and also

to the protoplasm of the neighboring cells, and that something we chose to call activity or excitation, in spite of the fact that either of these terms, in some instances at least, is a misnomer. It would be better if we could use some algebraic symbol. Practically every other biological term that we might use has various connotations which make it just as unsatisfactory. We must therefore divorce ourselves from the uncertainties of word disagreements, and decide to call activity of streaming plant protoplasm the state in which movement ceases\*. This state is produced by the electric current and by mechanical pressure. It is also produced in various other ways, but as has already been stated, we shall avoid completeness in order to make our argument more forceful even if less extensive.

The activity or excitation produced by the electric current or by mechanical pressure is prevented by anesthetics. In solutions of ether of about 1 to 2 percent, there is no longer any effect of pressure and no longer any effect of the electric current (compare LAUTERBACH '21, KÔKETSU '23).

In nerve and muscle cells the living substance reacts to exactly the same agents that the plant protoplasm does. When nerve and muscle cells are exposed to the action of an electric current, they are thrown into a state of activity. The muscle cell contracts, and an impulse passes along the nerve fiber. Again, both in the case of the muscle cell and the nerve cell, activity is prevented by the addition of ether in approximately 1 to 2 percent concentration.

There is thus an absolute correspondence between the conditions which cause activity of muscle and nerve and those which cause what we have chosen to call activity of streaming plant protoplasm (compare also HÖRMANN '98). We might show moreover that other types of protoplasm are also acted upon in somewhat similar fashion. When a muscle cell responds to a stimulus, it contracts. But for the usual type of cell, practically the only type of physical response possible is division. Various agents

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\* HÖRMANN '98 has developed a similar concept. For this worker, electrical stimulation ("Erregung") of streaming plant protoplasm results in a cessation of movement. Here too the term stimulation is not used in the sense in which it is ordinarily used by students of protoplasmic streaming.

cause cells to divide. Electric currents and mechanical forces are not the best means, but they have sometimes proven effective (see for example LILLIE and CATTELL '25, MATHEWS '01). Moreover division is prevented by 2 percent ether. Or we might take the case of ameba. Its normal state is a state of motion in which the cell has various pseudopodia extended. Electric currents and mechanical pressure or shock cause a retraction of pseudopodia.

To any one who has surveyed the whole field, there can be no question but that in many types of protoplasm, mechanical pressure and electric currents, and some other agents which we have not chosen to consider, cause a definite activity or response of the protoplasm, and this response is generally prevented by low concentrations of ether or other fat solvent. The point that we should now like to make is that in all these types of protoplasm, the effect of the electric current and the mechanical pressure on the one hand, and of the fat solvent on the other, is the same. To the student of general physiology this may seem almost a truism. To the reader of this book it must be obvious that in many types of living substance the electric current and fat solvents have been found to produce the same effect, in the one case or the other, wherever this effect has been tested. In an earlier chapter, it was shown that electric currents produce gelation or coagulation, or if one pleases, a great increase in protoplasmic viscosity, in ameba, in *Tradescantia*, in *Phaseolus*, in *Cosmarium* and in man. Mechanical pressure or injury causes a similar change in the protoplasm of *Vicia*, *Phaseolus*, *Secale*, *Raphanus*, *Tradescantia*, and *Arbacia*. Low concentrations of ether were found to have the opposite effect, that is to say a decrease in protoplasmic viscosity, in *Vicia*, *Callisia*, *Reticularia*, *Spirogyra*, *Elodea*, *Arbacia*.

The evidence as far as it goes is concordant. Whenever the action of mechanical pressure or injury has been tested, or whenever the action of an electric current has been tested, both of these agencies have been found to produce a great increase in the viscosity of the protoplasm. And whenever the action of dilute concentrations of fat solvents has been tested, it has been found that they cause a decrease in protoplasmic viscosity.

The reader by this time will probably perceive whither the argument leads. It is our aim to attempt an explanation of the more complex and less readily observable forms of protoplasmic



activity on the basis of what is known for the simpler types of activity. This may seem a logical procedure, but it is against all precedent. Heretofore the physiology of muscle and nerve has usually been studied and explained primarily by men trained in medical physiology. These workers are for the most part not very sympathetic with the protoplasm concept, and they have little or no interest for morphological study. But if it is true that muscle and nerve in their behavior toward various agents are exactly like various other more simple types of protoplasm, it can scarcely be doubted that the study of these simpler types of protoplasm will yield results of importance for the understanding of muscle and nerve physiology.

Objection may be made, and with justice, that the protoplasm of muscle and of nerve is very different from the more simple types of living substance on which our viscosity data are based. This we must admit. But on the other hand, in the case of muscle for example, it has never been proven that the contractility of the muscle protoplasm depends on the fibrils or striations that can be seen in it. It is probably true that all muscle fibers contain fibrils. And it is only logical to suppose that these fibrils are responsible for the contractility. Against this point of view is the fact that in its behavior toward the electric current, toward mechanical pressure, and toward various other stimulating agents, muscle protoplasm reacts in exactly the same way that the simpler types of protoplasm do. And it reacts in the same way toward ether. In view of these facts, we should like to present as an alternative point of view that the fibrils in muscle protoplasm are not primarily concerned with the process of contraction. This we realize is counter to the opinion of practically all authorities on muscle physiology, present and past. But in view of the fact that muscle physiologists after so many years of intensive study have failed to present a plausible theory of muscular contraction from a physical or colloid chemical standpoint based on the presence of fibrils, it is after all at least permissible to entertain the notion that the fibrils are not primarily responsible for the initiation of the contraction. Moreover, there is some direct evidence in support of this view.

As every student of embryology knows, the heart of the chick begins to beat early in the second day of incubation. The muscle cells which cause this beat have no fibrils. Fibrils can

only be demonstrated in these cells on the third day of incubation. This was shown by ECKHARD '67 and later by CHIARUGI '87. MARCEAU '03 showed in the embryos of the lizard *Anguis fragilis*, that at a time when the heart beat clearly, no fibrils could be discovered in the protoplasm of the muscle cells. In a 11 mm. frog tadpole which moved rapidly and must therefore have had a circulation, MARCEAU was also unable to detect any fibrils in the cells of the heart. It is to be noted that MARCEAU used elaborate modern histological methods. We may therefore regard it as proven that in vertebrate embryos generally the muscle cells of the heart can function without fibrils. Similar results have been obtained by the students of tissue culture (see for example LEWIS '23a).

It might be argued that fibrils are really present in these muscle cells, but that they are invisible. This is hardly a valid argument, for it is the presence of visible fibrils that we are denying. If ultramicroscopic fibrils were present, this would be a different matter from a physical point of view.

Even though we present the view that fibrils do not originate the muscular contraction, it must not be thought that we deny that they play a part in the dynamics of the muscle cell in which they are found. It is perhaps true that in some muscle cells there are fibrils which by their elasticity cause an expansion following contraction. It is also possible that in some cells there are fibrils which exert a tension on the cell and tend to pull it together, that is to say to contract it. The presence of such fibrils would not in the least interfere with our general scheme of interpretation. A muscle cell is doubtless acted upon by various forces, some of which tend to make it longer and some shorter. We can readily conceive that among the forces which tend to shorten the cell may be the tension exerted by fibrils. What we should like to deny is that this fibrillar tension is a rapidly variable factor in ordinary muscular contraction; or to be specific, that it can be made to develop suddenly by an electric current or by mechanical pressure.

It seems a fair assumption that the activity or the contraction of a muscle cell without fibrils depends on the same physical principles that it does in a muscle cell with fibrils. If we conceive of such a muscle cell without fibrils as being exposed to an electric current of sufficient strength to induce contraction, it would be hard to believe that the protoplasm of this cell would not

react to the electric current in the same manner that all other types of protoplasm which have been investigated have been found to react. And we believe also that the behavior of such a cell toward ether would not differ from the behavior of all the other types of cells that have been studied. An actual test of these statements would probably not be very hard to make. LEWIS '23a has described the presence of Brownian movement in smooth and striated muscle cells in tissue culture. It would only be necessary to send an electric current through these cells and to determine whether or not the amplitude of the Brownian movement was affected. Similarly one could study the action of ether and other fat solvents.

It should be stated that BIEDERMANN '09 has offered some interesting evidence in support of the view that contraction of a muscle cell is accompanied by a coagulation of its protoplasm. BIEDERMANN states that in the transparent muscle of some invertebrates there is a wave of opacity which follows along the course of the contraction. Thus the retractor muscles of *Sipunculus nudus* are a clear transparent blue in the resting condition, but at the least sign of contraction, the contracted region becomes white and opaque. He records similar changes in snail muscles.

In present-day muscle physiology there is no plausible explanation of how an electric current or mechanical pressure can cause muscular contraction by acting on the fibrils of muscle cells. For a time there did seem to be a plausible explanation. ENGELMANN found that a violin string shortened in the presence of acid. Following this experiment, it was commonly believed by many physiologists that the production of acid in muscle cells led to a shortening of fibrils in the same way that acid led to the shortening of a violin string. This view has now been generally abandoned for a number of reasons, chief among which is the fact that the violin string shortens only if it is composed of twisted fibers. The individual fibers do not shorten (see BERNSTEIN '15). We can scarcely assume that muscle fibrils are composed of twisted elements.

Mechanical pressure and the electric current do actually cause coagulation or gelation in many types of protoplasm. If they have the same effect on the muscle protoplasm, we are in a way toward the understanding of the physical aspects of muscular contraction.

On ordinary proteins or on inanimate colloids generally, neither mechanical pressure nor an instantaneous electric current has any coagulative action. How then shall we interpret the coagulative action that these agents have on living protoplasm? It has already been brought forward in chapters 8 and 14 that the electric current frequently causes the appearance of vacuoles both in plant and animal protoplasm. Mechanical pressure was found to have the same effect. To anyone who has followed the discussion in chapter 14, there is good evidence that the coagulative or gelatinizing action of the electric current depends primarily on the reaction which we have found of so frequent occurrence in living systems, and which shows certain resemblances to blood clotting. This reaction, it will be remembered, depends first on the presence of free calcium. The calcium unites with some substance which may be found in granules, and a thrombin-like compound is the result. This thrombin-like compound then produces a coagulation which frequently takes the form of films or vacuoles, but does not necessarily take this form. When pigment is present, the reaction is accompanied by a loss of this pigment.

There is actually some evidence that the reaction that we have just outlined can occur and does occur. The evidence is perhaps not very good. No one has ever studied the problem of muscular contraction from this standpoint, and we shall have to depend on one or two chance observations in the literature. We shall take up first some relatively recent studies of R. S. LILLIE on *Arenicola* larvae, and then some old observations of KÜHNE.

In a series of studies of the larva of the annelid *Arenicola*, R. S. LILLIE '09, '11 b, '12, '13 showed that violent contractions of the musculature were always accompanied by the loss of a yellow pigment. LILLIE himself saw the resemblance between this phenomenon and the cytolysis so often described for sea-urchin eggs (see LILLIE '12, p. 380, footnote). But for LILLIE, cytolysis and loss of pigment were all manifestations of an increase in permeability of the plasma membrane. That loss of pigment has nothing to do with changes in permeability is certainly true in the case of the *Arbacia* egg, where the process of pigment release from the pigment granule can be studied outside of the cell in the total absence of any permeable or semipermeable membrane. This is a point that has already been considered

(see p. 246). What we wish to emphasize here is that LILLIE found in the muscle contraction of worm larvae, evidence in muscle cells of the reaction which occurs so widely in other types of protoplasm. In the presence of fat solvents in anesthetic concentration, the muscle cells did not contract and no pigment was given off. LILLIE attributed the action of the anesthetic to an effect on the plasma membrane. But it can be shown in the case of the sea-urchin egg, that low concentrations of ether tend to prevent an escape of pigment from the pigment granule when this is outside of the egg and not surrounded by any plasma membrane (unpublished experiments). However, we must not lose track of the main point, that in the contraction of at least one type of muscle cell, there is evidence that a reaction occurs exactly comparable with the reaction which we have described in Chap. 14.

The observations of KÜHNÉ '64 are even more interesting. KÜHNÉ describes the preparation of a muscle plasma from frog muscle fibers, and this muscle plasma outside of the cell was found to give a reaction which might easily be interpreted as being akin to that found in the protoplasm of intact cells. In order to appreciate the significance of KÜHNÉ's observations it will be necessary to describe his methods of work carefully. Ordinarily if one cuts or squeezes a muscle fiber no fluid emerges, or if fluid emerges it travels only a very short distance before a pronounced surface precipitation reaction sets in. In order to press out his muscle plasma, KÜHNÉ worked under the very conditions which would prevent a surface precipitation reaction. From the discussion in Chap. 13, these conditions are known to be: first, low temperature; second, the absence of calcium; and third, the absence of thrombin-like substances. In describing his experiments, KÜHNÉ states: "Schneidet man die Muskeln der Unterschenkel von 8—12 durch Injection mit einprozentiger Kochsalzlösung von Blut gereinigten Wasserfröschen einzeln, ohne sie an ihren Oberflächen zu verletzen, herunter, so kann man sie zu einem kompakten Haufen vereinigt bei der bezeichneten Temperatur in etwa 3 Stunden in einen zusammenhängenden festen Eisklotz verwandeln."

The temperature in question was  $-7$  to  $-10$  degrees C. and the muscles were cut with cold knives and ground in a cold mortar. In studying the description of KÜHNÉ's experimental proce-

ture, it is interesting to note, first that he worked at very low temperatures, second that he washed the muscles free of calcium by injecting the frogs with sodium chloride solution, and thirdly that he was careful not to injure the muscle. Injury of the muscle might very well have resulted in the production of substances similar to the ovothrombin described in Chap. 13. Note too that there was no blood and hence no thrombin present. All in all, if KÜHNE had known the exact details of the surface precipitation reaction as we have described it, he could not have chosen his methods of work better in order to avoid it.

The muscle plasma prepared by KÜHNE is fluid in the cold, but forms a coagulum at room temperatures. This is of course to be expected. Sea-urchin eggs torn in the cold form a precipitation film as soon as the temperature is raised. If a drop of the muscle plasma is allowed to fall into distilled water, a coagulum forms immediately. This may be due to a reaction like that of protoplasm toward distilled water, but is more probably due to an action of the distilled water on the globulins of the muscle plasma. But for us the most important experiment of KÜHNE's is that described on page 25 of his book. He divided some ground-up muscle ice or plasma into two parts and to one part he added frozen coagulated blood of the frog, the other part serving as a control. Both samples were filtered at 0°, and the time of coagulation compared as the temperature rose. In the presence of blood, a coagulation of the muscle plasma occurred much more rapidly than when the coagulated blood was absent. It is thus evident, if KÜHNE's experiment is correct, that the thrombin of frog blood causes, or at least hastens, the coagulation of the muscle plasma. So too KÜHNE interpreted his experiment.

Thus, apparently, in the protoplasm of the frog's muscle there is a material whose coagulation is favored by the presence of thrombin. If this experiment of KÜHNE's can be repeated, it is very strong evidence that in muscle a reaction may occur which is similar to the reaction which occurs during the coagulation of blood.

It should be noted that KÜHNE's experiment was made at a time when the importance of thrombin in blood coagulation had already been demonstrated, but when it was still not known that calcium salts played any part in the process. If KÜHNE had known of the role of calcium salts in blood coagulation, he would certainly have studied the behavior of oxalated muscle plasma.

It would be very interesting to find out if calcium salts play any part in the coagulation of muscle plasma. But even as it stands, KÜHNLE's experiment shows that muscle protoplasm, that is to say the sarcoplasm, can undergo a reaction like that described in Chap. 14, a reaction which we have shown to be of very wide occurrence in both plant and animal cells.

There are three very important aspects of protoplasmic activity. One is that protoplasm is made active by electric currents, by mechanical pressure or injury, and by various other so-called stimuli. It has been shown that the electric current and mechanical injury both cause a coagulation of the protoplasm, and there are reasons for believing that the coagulation is due to the type of reaction we have so often considered. Secondly, fat solvents in dilute concentration prevent protoplasmic activity without killing the protoplasm. This effect of fat solvents has been shown to be related to the colloid chemical behavior of the protoplasm in the presence of these reagents. Moreover, as has been mentioned previously, experiments are in progress which may throw some light on the manner in which the fat solvent produces its effect in those cells in which there is an easily observable internal precipitation reaction. A third aspect of protoplasmic activity, and one we have not as yet considered at all, is the fact that whenever protoplasm is made active, the effect tends to be transmitted from one part of a cell to another, and from one cell to another. This is an extremely important aspect of protoplasmic activity, and we shall therefore attempt to find whether our point of view offers any clues as to the possible mechanism of the transmission of activity.

The subject of transmission of activity has been studied mostly in nerves. In spite of the fact that very many investigators have attempted to understand the transmission of the nervous impulse, it must be admitted that our present theories are scarcely very convincing. They rest almost entirely on analogy. The transmission of the nervous impulse is compared with one or another phenomenon in the inanimate world. To the student of the colloid chemistry of protoplasm, this use of analogy must seem of somewhat doubtful value. It so happens that in spite of many attempts of various workers to draw conclusions regarding protoplasm from the behavior of gelatin gels and other materials supposedly similar to the living substance, this method

of attack has never yielded a single truth which could be confirmed when the protoplasm itself was studied. Perhaps the present-day theories of the transmission of the nerve impulse will prove to be true, but at present they are little more than guesses.

It would hardly be worth our while to consider every type of protoplasmic transmission from the standpoint of the colloid chemistry of protoplasm. We shall discuss chiefly the transmission of activity in plant protoplasm. It has long been known that when a plant tissue is cut, there is a reaction or change that travels out from the surface of the wound. Actually there are three aspects of this phenomenon. In the first place, it was noticed that the nuclei in cells near the wound moved in a direction toward the wounded surface (see TANGL '84, NESTLER '98, NĚMEC '01, KARLING '26), and this movement passed as a wave out from the wounded surface. Secondly, there is a coagulation wave, or at any rate a wave of increased protoplasmic viscosity that travels out from the cut surface to the neighboring cells (see BUENNING '26a, b, see also p. 128). Thirdly, as shown clearly by NĚMEC '01, there is a wave of vacuolization. In the time following the cut or wound, the zone in which the protoplasm shows vacuolization phenomena becomes progressively greater. Doubtless the movement of nuclei, the coagulation, and the vacuole formation, are all different aspects of the same reaction which travels out from the cut surface. The evidence is strong that this reaction is the same as the one defined in Chap. 14. The fact that there is a marked increase in protoplasmic viscosity, that there is moreover a vacuolization, is certainly a strong indication that this is the case.

We have no desire to generalize from this one instance. We should like to point out, however, that if protoplasmic activity does really depend on the type of reaction which we have postulated, then there is a conceivable mechanism for the transmission of protoplasmic activity from one part of a cell to another, or from one cell to another. It will be remembered that the reaction occurs in three stages, of which the first is the liberation of free calcium, the second the union of this calcium with other materials to form a thrombin-like substance which we have called ovothrombin in the case of the sea-urchin egg, and the third the reaction between this thrombin-like substance and another substance to form a precipitation product. The ovothrombin of the sea-ur-



chin egg was found to be surface active. Hence it might travel very rapidly over surfaces, for BRINKMAN and v. SZENT-GYÖRGYI<sup>23</sup> have shown that surface active substances can travel over surfaces at the rate of 25 cm. per second, or even faster. More important than this is the fact that there is evidence that the third stage of the reaction sets free calcium (see p. 227). If this is the case, the reaction may be self propagating, just as the reaction which travels along a train of gunpowder is self-propagating.

It seems probable that the wave of reactions that we have postulated is in one way or another related to the wave of electric negativity which accompanies the activity of many types of living substance. In view of the importance of calcium in these reactions, it is interesting to note that, according to FEENSTRA '26, there is no current of action in a frog retina perfused with calcium-free Ringer solution. These currents return when calcium is again added to the perfusing solution. Further it should be noted that we have frequently stressed the similarity between the reactions which occur when protoplasm is injured and when it becomes active. On the basis of this similarity it is easy to understand why the surface of injured and active protoplasm shows the same electrical behavior.

In this chapter an attempt has been made to establish a point of view regarding the usual type of protoplasmic activity. That such a point of view is desirable is indicated by the fact that the same physical agents induce activity in widely different types of living substance, and that this activity may be prevented by a single reagent which may be used in almost the same concentration both for plant and animal cells. It has certainly been shown that in all those cases in which investigation has been made the agents which produce activity cause a coagulation, or at any rate a great viscosity increase, in the protoplasm, and that most of the reagents which prevent activity have the opposite effect. From this certain information it has been argued that in other types of protoplasm in which as yet no viscosity measurements have been made, activity is also due to coagulation processes and anesthesia in general to a prevention or reversal of coagulation. Furthermore, an attempt has been made to inquire into the nature of the coagulation process, and some evidence has been gathered which indicates that the reaction involved may be the specific reaction which was considered in detail in Chap. 14.

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The following list is far from a complete one. No attempt has been made to cite every paper which has a bearing on the colloid chemistry of protoplasm, for in the broadest sense there is no branch of biology which does not have some relation to the subject. In general, purely chemical or physical references have not been included. Many papers cited in the bibliography are not mentioned in the text. This is sometimes due to the fact that the subject of the paper did not fit in with the main thread of the discussion. In other instances papers have been included which the author found barren. It was thought that others might be able to glean valuable information where he had failed to find anything of interest. All papers were consulted directly with the exception of those marked with an asterisk (\*).

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